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ANNUAL REPORT 7
JULY 1, 1976 THROUGH SEPTEMBER 30, 1977

NINCDS

PART I

NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

ANNUAL REPORT of program activities

JULY 1, 1976 THROUGH SEPTEMBER 30, 1977

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OFFICE OF THE DIRECTOR

Office of Program Planning and Evaluation

N01 NS-6-2345 - JRB Associates, Inc.
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Office of Biometry and Epidemiology

N01 NS-4-2321 - Massachusetts General Hospital
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N01 NS-6-2337 - The University of Newcastle Upon Tyne
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N01 NS-4-2335 - National Analysts, Inc.
Survey of the Incidence, Prevalence, and Costs of Multiple Sclerosis 13-d

N01 NS-4-2334 - Research Triangle Institute
Survey of the Incidence, Prevalence and Costs of Head and Spinal Cord Injury 15-d

N01 NS-4-2336 - Westat, Inc.
Survey of Intracranial Neoplasms 17-d

N01 NS-6-2333 - Westat, Inc.
Nationwide Study of Stroke 18-d

Y01 NS-70031 - U. S. Bureau of the Census
N01 NS-7-2357 - University of Mississippi
Survey of Major Neurological Disorders in Copiah County Mississippi 20-d

RFP-NIH-NINCDS-77-05 - Contractor to be Selected
Y01 NS-7-0030 - National Center for Health Statistics
Comprehensive Disease Statistics Survey 22-d

N01-NS-3-2304 - Univ. of Texas at Houston, School of Public Health
Stroke Risk Factors in Panama 24-d

PH-43-64-44 - National Academy of Science
Epidemiologic Study of Amyotrophic Lateral Sclerosis and Other Motor Neuron Diseases 25-d

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Infectious Diseases Branch

N01 NS-4-2308 - University of Wisconsin
Study of the Use of Zonal Ultracentrifugation and Affinity Chromatography in the Etiology and Diagnosis of Multiple Sclerosis 9-ee

N01 NS-4-2309 - Scripps Clinic and Research Foundation
Detection of Viral Genomes in Human Neurological Diseases 10-ee

N01 NS-5-2311 - The Joseph Stokes, Jr. Research Institute of the Children's Hospital of Philadelphia
Immune Electron Microscopic Techniques as an Aid for Determination of the Etiology of Multiple Sclerosis 11-ee

N01 NS-5-2309 - Mount Sinai School of Medicine
Virological Studies in Parkinson's Disease 12-ee

N01 NS-5-2318 - University of Florida
The Viral Induction of Malformations in Developing Rhesus Monkeys 13-ee

N01 NS-2-2306 - Meloy Laboratories
Herpes Virus Induction of Cervical Cancer in Cebus Monkeys 14-ee

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Office of Director

NIH-NINCDS-72-2300 - Clinical Neurol. Info.Center, Univ. of Nebraska
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Developmental Neurology Branch

N01 NS-2-2303 - University of Wisconsin
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N01 NS-2-2321 - Johns Hopkins University
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N01 NS-2-2322 - Boston University
Maternal Drug Ingestion and Fetal Abnormalities 14-hh

N01 NS-3-2311 - Pennsylvania State Univ, M.S. Hershey Medical Center
Analysis of General Pathology and Placental Pathology of the Collaborative Perinatal Project Data 15-hh

N01 NS-3-2312 - Children's Hospital Medical Center, Boston, MA
Combined Neuropathologic and Epidemiologic Study 17-hh

N01 NS-3-2320 - Beth Israel Hospital, Boston, MA
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N01 NS-4- 2326 - University of Minnesota
Analysis of Speech, Language and Hearing Deficits to Facilitate Prevention, Diagnosis and Treatment 19-hh

N01 NS-5-2308 - University of Michigan
Physical Growth Analysis 24-hh

N01 NS-5-2326 - University of Colorado
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Epilepsy Branch

N01 NS-8-1310 - New Castle State Hospital
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N01 NS -9-2196 - Univ. of Virginia School of Medicine
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N01 NS-0-2281 - University of Washington
Complex Partial Seizure Drug Studies 9-ii

N01 NS-1-2282 - University of Washington
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N01 NS-2-2313 - University of Kansas Medical Center
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N01 NS-3-2303 - Excerpta Medica Foundation
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N01 NS-3-2322 - Stanford Research Institute
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N01 NS-5-2302 - University of Utah
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N01 NS-5-2327 - Univ. of Minnesota
N01 NS-5-2328 - Good Samaritan Hospital, Portland
N01 NS-5-2329 - Univ. of Virginia Medical Center
N01 NS-6-2340 - Medical College of Georgia
N01 NS-6-2341 - Univ. of Washington
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N01 NS-6-2342 - Methodist Hospital - Houston
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N01 NS-6-2347 - North Carolina Heart Association
N01 NS-6-2349 - Trustees of Boston University
N01 NS-6-2350 - Rush-Presbyterian-St.Luke's Medical Center
N01 NS-7-2348 - Univ. of Oregon Health Sciences Center
N01 NS-7-2351 - Univ. of Rochester
N01 NS-7-2354 - Virginia Commonwealth Univ.
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N01 NS-4-2306 - Univ. of Minnesota
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N01 NS-4-2328 - Cornell Univ. Medical College
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N01 NS-5-2316 - Univ. of Pennsylvania
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N01 NS-5-2332 - Univ. of New Mexico
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N01 NS-6-2346 - National Academy of Sciences Medical Follow-Up Agency
Feasibility Study, Phase, I, Follow-up Study of Vietnam Registry of Head and Spinal Cord Injuries 18-jj

N01 NS-7-2361 - Yale University
Feasibility Study to Develop Data Collection Instruments and Protocols at the National Acute Spinal Cord Injury Centers 19-jj

N01 NS-7-2358 - University of Maryland
Functional Recovery in Rats after Spinal Cord Lesion 20-jj

N01 NS-6-2116 - Massachusetts Institute of Technology
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Y01 NS-4-0019 - VA Hospital, Minneapolis, MN
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N01 NS-4-2323 - City Univ. of New York Graduate Center
Prescriptive Fitting of Wearable Master Hearing Aids 27-kk

N01 NS-5-2313 - Univ. of Florida
Study of Auditory Sensitivity and Discrimination in Young Children 28-kk

N01 NS-5-2317 - Univ. of Pittsburgh
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N01 NS-5-2322 - Regents of the Univ. of California
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N01 NS-5-2323 - John F. Kennedy Institute
Study of Sensory and Perceptual Functioning of Young Children With and Without Delayed Language Development 33-kk

N01 NS-5-2324 - Special School District of St. Louis
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N01 NS-5-2331 - Evaluation of Subjects Presently Fitted with Implanted Auditory Prostheses (Eye & Ear Hosp. of Pittsburgh) 36-kk

N01 NS-6-2353 - Wayne State University
Evaluation of Procedures for Screening Preschool Children for Signs of Impaired Language Development 37-kk

Y01 NS-7-0033 - Naval Research Laboratory
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N01 NS-0-2275 - Huntington Institute of Applied Medical Research
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N01 NS-0-2276 - Massachusetts General Hospital
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N01 NS-0-2279 - University of Rochester
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N01 NS-1-2286 - University of Florida
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N01 NS-4-2304 - Bowman Gray School of Medicine
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N01 NS-3-2306 - Univ. of California at Los Angeles, Brain Information Center (UCLA-BIS)
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N01 NS-5-2306 - Stanford University
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N01 NS-3-2307 - University of California, San Francisco
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N01 NS-3-2313 - EIC Corporation
Safe Procedures for Electrical Stimulation of the Nervous System 18-ll

N01 NS-2-2314 - Case Western Reserve University
Study of Intramuscular Electrical Stimulation of Muscle 19-ll

N01 NS-3-2319 - Indianapolis Center for Advanced Research (ICFAR)
Study and Test Ultrasonic Techniques for Diagnosis of Cerebral Disorders 20-ll

N01 NS-4-2331 - University of California, Los Angeles
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N01 NS-4-2332 - University of Minnesota
Study of the Effects of Electrical Stimulation of the Cerebellum 23-ll

N01 NS-6-2343 - Massachusetts Institute of Technology, Neurosciences Research Program
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N01 NS-6-2344 - McMaster University
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National Institute of Neurological and Communicative Disorders and Stroke
July 1, 1976 through September 30, 1977

NOBEL PRIZE 1976

For the NINCDS this past year has been a very mixed year. It was during this year that the Institute was especially distinguished by the selection of one of its staff, Dr. D. Carleton Gajdusek, as the 1976 Nobel Laureate in Medicine or Physiology. He joins three other NIH intramural scientists, Nirenberg, Anfinsen and Axelrod, at this pinnacle of scientific distinction. Although this is a first for NINCDS, it is noteworthy that three of the four at NIH are currently engaged in research on the nervous system. In fact in recent decades a clear majority of the Nobel prizes in physiology or medicine have been conferred on investigators in the neurosciences. We are particularly pleased at Gajduseks's selection since it highlights the previously neglected field of virology and because it recognizes his dual discoveries: that chronic degenerative disorders of the human central nervous system can be caused by transmissible agents and that the slow virus-like agents that cause human kuru and Creutzfeldt-Jakob disease, as well as scrapie of sheep and rodents and transmissible mink encephalopathy, are so atypical in properties, interaction with host tissues and survivability that they appear to represent a totally new class of microbiological agents. Some of us were privileged to be present in Stockholm last December 10, to witness the impressive ceremony of the Nobel awards and to express our great affection and pride for our distinguished colleague. As all who know him can attest, Carleton Gajdusek is not only a brilliant scientist-clinician but also a very talented and warm human being.

PERSONNEL CHANGES

During this same year we were confronted by some notable non-accomplishments. With the help of our diligent search committee we came very close to selecting a new chief of our intramural Surgical Neurology Branch, but the discrepancy between Federal ($47,500) and private sector salaries ($65,000++) for academic neurosurgeons has so far proved to be an insurmountable stumbling block. The search committee and our intramural staff continue to address the problem, but after two years the likelihood of success seems much diminished.

Likewise, we have been frustrated in our desire, in concert with our SIPAC Committee, to select the best possible successor to Mrs. Ruth Dudley, who retired in June after 19 years as chief of the NINCDS office of Scientific and Health Reports. As we approached the time for a selection decision, we encountered the constraints recently imposed by the DHEW Special Placement Program. We still await a decision on our appeal for a waiver to disregard the SPP lists and to go forward with our selection. Now is a particularly critical time for the NINCDS in terms of informational and public relations activities. Thus, we look upon the filling of this post as particularly sensitive and important. The delay in appointing a successor, now approaching six months can hardly be helpful.

Other personnel changes have been mixed as well. The NINCDS is already enjoying the appointment of two key staff members during this year. Dr. Kenneth Hisaoka has become the first Director of the NINCDS Extramural Activities Program and thus completes the reorganization of our extramural programs. This year represents the first full operational year for our reorganized extramural programs, and although there remain some details to be dealt with, I think it fair to say that the new look is working well.

The other key appointment was that of Mr. Richard Sherbert as Executive Officer, to replace the previous incumbent who left for NIEHS after only a year in the post. It is difficult, at best, for an institute to change executive officers but to make two such changes in a little over twelve months can prove most disruptive. It is to Dick Sherbert's credit that the transitional disruption was minimized and that much needed attention to major problems is being promptly instituted.

One sad note for all of us was the unexpected death from a rapidly progressing cancer of Dr. Michelangelo Fuortes, beloved chief of the NINCDS intramural Laboratory of Neurophysiology. Mike Fuortes was not only a scientist and teacher of international renown but also a socially concerned citizen. To lose him at the height of his career is indeed a tragedy for us all. When he knew he was dying he urged on us a worldwide search for his successor as lab chief and helped us set up the search committee. A distinguished search committee is now in the process of reviewing candidates for recommendations to us.

ADMINISTRATIVE PROBLEMS: FCRC BLDG. 376

Several major administrative problems still elude satisfactory solution. Perhaps the most pressing and most frustrating is the continued failure of the Litton Bionetics contractors at the Frederick Cancer Research Center to provide an acceptably renovated Building 376 for occupancy by the atypical slow virus research program of the intramural Laboratory of Central Nervous System Studies under Gajdusek and Gibbs. Currently the FCRC segment of this program is housed in space loaned by the NCI, which badly needs the space back for their own programs. The renovations to Bldg. 376 at a cost of over $1.5 million are intended to provide laboratory, quarantine and animal holding facilities at P-2 and P-3 levels for scrapie, kuru and Creutzfeldt-Jakob disease research, including isolation, purification and characterization of the scrapie agent.

After two years of renovation, the building was to have been ready almost a year ago (in December 1976). Air flow and differential pressures (balance) could not be satisfactorily corrected. After months of desultory attempts at corrective efforts, it became obvious that there were huge leaks through the building wall, open ducts walled up behind partitions, collapsed and undersized ducts, generally badly leaking duct joints, and much more--all of which should have been taken care of by the original contractor and should have been obvious to the contract inspectors. At considerable additional direct cost and incalcuable loss to the research program, progress is finally being made in correcting the deficiencies, but we still have no clear date for occupancy. Despite the fact that the NINCDS will occupy this building and be responsible for research that must be carried out with P-2 and P-3, perhaps even P-4, precautions, the institute has been excluded from much of the considerations of the problems with the building. This seems to me to be wholly unreasonable and irresponsible.

CLINICAL CENTER BEDS

Another chronic intramural problem persists as this Institute attempts to make optimal use of the small number of research beds allocated to the NINCDS. This is a complex problem. On the one hand it is related to the availability of central services (nurses, orderlies, etc.) from the NIH Clinical Center, such that NINCDS bed occupancy has, on occasion, been restricted to less than 70 percent of our 52-bed capacity. In contrast to several other NIH institutes, whose normal bed occupancy runs lower than that, the NINCDS has continued to operate at 90-100 percent of capacity (when permitted), even borrowing beds from other institutes to meet our clinical research demands. On the other hand the bed problem also relates to the response of NINCDS intramural clinical research to recommendations of our external advisors to diversify and the consequent burgeoning of our clinical research programs.

From an original divison of 26 beds each to surgical and medical neurology branches, the current usage (not allocations) is the following: Experimental Therapeutics Branch, Therapeutics Section 5; Pharmacology Section 5; and Clinical Epilepsy Section 5; Surgical Neurology Branch 7; Clinical Neurosciences Branch, Functional Neurosurgery Section 4; Neuroimmunology Branch 4; Developmental and Metabolic Neurology Branch 6; Infectious Diseases Branch 0; Medical Neurology Branch (neuromuscular diseases) 16. It is clear that imbalances still remain to be redressed and that consideration must be given to the impacts of a new neurosurgical program (if a chief is recruited), of the NIH Ambulatory Care Research Facility (ACRF) now under construction, and of a planned clinical communicative disorders branch. Hopefully the ACRF will at last make it possible to restrict inpatient beds to appropriate research patients, with a shift in many current inpatient studies to outpatient programs. However, the NINCDS bed problem is part of an NIH-wide problem, wherein some services exhibit significant and chronic under-utilization of their beds while NINCDS runs at 90 to 100 or more percent and could easily use more. Better procedures for allocation and utilization of NIH bed resources are desperately needed.

PERSONNEL CEILINGS

The final major and chronic administrative problem concerns our other major resources--personnel slots and budget. The NINCDS has been more harshly dealt with in terms of personnel ceilings and costs than all but one other NIH institute. During FY 1976 we finally began what promised to be a slow return toward former levels when the Congress mandated increases for all the NIH. Thus, at the start of FY 1977 the NINCDS stood at 543 full-time permanent (FTP) positions, up from a low of 511 a year ago. However, this year we were again cut back to 522--at a time when all programs were already squeezed unmercifully. Our Intramural Program has yet to recover an adequate research or clinical associate program and will have extreme difficulties in mounting an effective surgical neurology program and in staffing the FCRC slow virus facility. We have had to house and staff two national commissions (Epilepsy and Huntington's Disease) with no funds or slots provided. This has meant postponement of recruitments and of funding worthwhile research (with the $1.5 million that the commissions have cost). As the complexities of grant and contract supported research increase yearly, our staffing has been unable to keep pace and we lack key program personnel and backup staffs.

It makes little sense to charge us with a $155.5 million research program and then deny us $0.5 million for needed additional management personnel or question the expenditure of a like amount for travel of advisors, site visitors and staff to monitor and oversee the program. We are bombarded with mounting demands for reports and analyses and with increasing numbers of regulations for which we simply do not have the staff to respond. In this regard middle sized institutes like the NINCDS are very much like middle income citizens who are being stretched beyond the limits of their resources, being neither rich enough to buy what is additionally needed nor poor enough to receive the charity of their neighbors. It appears to us all too customary for the higher echelons to look upon all of us at NIH like NCI or NHLBI, each of which have maintained or increased their resources and started with far fuller staffing with which to respond to such administrative demands. As I contemplate contingencies, should a key staff member leave, I have no adequate back up staff to turn to and I see key staff inexcusably overworked.

UNDERFUNDING

This year (FY 1977) is the first in 4 years to be marked by any significant increase in the NINCDS budget. Over the past three years our budget has been stationary between $142.5 million and $144.4 million, despite significantly mounting inflation and costs of doing research. This year we received $155.5 million, an $11 million increase over FY 1976; under other circumstances this would have seemed relatively handsome, but a more sober examination of the facts indicates that this increase fell far short of correcting the accumulated "deficits" of the previous years. In constant 1974 dollars, the NINCDS should have had in FY 1977 not $155.5 million but at least $182 million just to maintain the level of research support existing in FY 1974. Clearly the NINCDS has lost ground. We can support less research grants and each of them now costs at least 50 percent more. Even with the increase received in this fiscal year, the NINCDS has been able to fund only about 25 percent of approved grant applications. The priority cut-off was 150, with only selected, program-initiative grants funded beyond that level. This austerity denies support to a whole host of excellent, well above average proposals. For an institute that depends so heavily for the forwarding of its mission on funding of a wide variety of fundamental research, it is obvious that the NINCDS is grossly underfunded.

RESEARCH PROGRESS

Nevertheless, a review of the attached annual reports from the various NINCDS components indicates solid, often exciting advances in our knowledge of the nervous system and in our understanding of its disorders. Some of these drawn from extra- and intramural programs are briefly noted here.

OBE SURVEYS

Valuable data are being obtained from the probability sampling surveys being conducted under contract for the Institute's Office of Biometry and Epidemiology (OBE). The surveys on head and spinal cord injuries and on brain tumors have been completed and are in preparation for publication. The data indicate just over one million persons alive in the U.S. with head and/or spinal cord injury, with a yearly acquisition rate of nearly one-half million new cases, and a total annual cost of over $3.0 billion. This is to be contrasted with the NINCDS current research expenditures for CNS trauma of about $13.5 million (or less than 0.5% of the current national cost). The problems are especially tragic since two-thirds of the victims are young, white males (below age 35). The data on brain tumors indicate 61,000 cases alive in the U.S. with brain tumors, with a yearly acquisition rate of 34,900 cases, 52 percent of which are secondary (metastatic) malignant tumors. The annual cost to the country is $875 million, contrasted with about one percent of that amount devoted to research by the NCI and the NINCDS. Other surveys on stroke, and multiple sclerosis are in progress and the one on epilepsy is in the design stage. In addition there is in the implementation stage a comprehensive disease statistics survey to be conducted on hospital discharge records (plus later surveys of ambulatory care physician data) in collaboration with the National Center for Health Statistics, the Bureau of the Census, the CDC and 7 other NIH institutes. This NINCDS-conceived project will develop incidence and prevalence data and annual trends on a large variety of disorders. Finally, our OBE has begun a survey of major neurological disorders in a stable population in Copiah County, Mississippi, where the number of blacks and whites in the population are equal. It will be the first epidemiologigal study on neurolgoical disorders in a black population, with direct comparability with a corresponding white population. These studies have been cited in some detail because they represent innovative pioneering efforts by our scientific staff to provide much needed data for research and for health services planning.

COMMUNICATIVE DISORDERS

In the NINCD'S Communicative Disorders Extramural Research Program (CDP) the current investment of research funds can be broken down approximately as follows: for ear, nose and throat disorders 8%; for studies on the vestibular system and its disorders 6%; for hearing research and disorders 48%; for speech and voice research and disorders 11%; for language research and disorders 3%; for auditory and non-auditory effects of noise 5%; for communicative aids 6%; and for other special senses (olfaction, taste, touch, etc.) 13%. In the areas of vestibular, speech and language disorders the investments are disproportionately small in comparison to the magnitudes of the problems. Because of the disappointingly small number of research grant applications in these areas, our CDP staff has placed priority on contract initiatives for these problems. Children with hearing, speech and language disorders also represent priorities for research. It is estimated that 6% of all school age children present speech pathology. New techniques, using pressure sensors, electromyography, laryngograph real-time microprocessors and spectrographs, and biofeed back techniques, promise to revolutionize research in these areas. Early evaluation of hearing loss in young children is being facilitated by electrocochleography, brain stem evoked potential responses, and auditory impedance testing. Studies of infantile autism suggest that it is not a symbolic or communication disorder but a disorder of auditory processing. Special attention is being given to development of preventive measures for otitis media and of appropriate selection criteria for tonsillectomies and adenoidectomies (the commonest surgical operation in the U.S. today). A definitive evaluation of all 13 available patients with cochlear implants was completed and published by our contractor at the University of Pittsburgh this past spring. This study indicates that for the profoundly deaf the cochlear implant may be a more useful and acceptable cuing device than other more traditional devices. Support provided by CDP for further experimental research and development of multiple electrode arrays will continue our emphasis on further evaluation of such auditory prosthesis potentialities.

NEUROLOGICAL DISORDERS

The Neurological Disorders Extramural Research Program (NDP) accounts for about 40 percent of our extramural research funding. Its funding is approximately distributed to the following research areas: disorders of early life 26.7%; disorders of aging 10.4%; convulsive disorders 21.8%; demyelinating and sclerosing disorders 13.1%; neuromuscular disorders 17.3%; infectious disorders 3.4%; neuroendocrine disorders 0.3%; learning, behavior and other senses 7%. Again it is obvious that imbalances exist, with needs for increased initiatives in areas of aging, infectious and neuroendocrine research.

Despite continued initiatives for research on multiple sclerosis (MS) and allied disorders progress in that area is painfully slow. Grant proposals relevant to ALS are still scarce, and all the recent claims for MS diagnostic tests or MS-specific agents could not be confirmed. A major role in these evaluations has been played by the NINCDS intramural Infectious Diseases Branch (IDB). For example, the claim by two groups to have demonstrated a transmissible agent in brains of MS patients led early this year to an overenthusiastic editorial in the British journal, Lancet, to the effect that the agent responsible for MS had at last been isolated and that appropriate preventive (vaccine) and therapeutic measures could be expected in due time. Alas, this was not to be so. When our IDB scientists tried to repeat the studies and failed, they sought collaboration with the original claimants who also were unable to reproduce their earlier results and who have now published a retraction of the earlier paper in the same journal, Lancet. There have been at least three other such failures to confirm reports on MS, so that the complexities of this demyelinating disorder still frustrate the onslaughts of an impressive array of some of the world's best research talent. However, the picture is not quite that bleak, since these researchers continue to provide us with a great deal of new information on the responses of the nervous system to infectious agents and to immune factors.

A major responsibility of the NDP area is the completion of analyses and publication of monographs for the Collaborative Perinatal Project data on 50,000 mothers and their offspring through 8 postnatal years. The analyses and monographs are rapidly nearing completion, and some specific results are already being highlighted. Among these results are correlations of congenital heart defects with maternal ingestion of estrogens during pregnancy; identification of risk factors and prognosticators for cerebral palsy; and demonstration of factors in infants with febrile seizures that are predictive for subsequent development of epilepsy. This last study already promises to radically alter the approaches to prophylactic therapy in these youngsters.

The epilepsy projects funded by NDP are flourishing. For example, the anticonvulsant drug development and screening program has now screened 1800 compounds from 22 commercial and 12 academic sources. Of these, 40 with novel structures appear promising enough for more detailed evaluation. At the same time an active project of chronic drug testing in primates fitted with continuous recording and sampling monitors is providing accurately quantifiable data that closely approximate the human situation. The NDP staff has been in the thick of the attempts to secure FDA approval to release sodium valproate for marketing in the U.S. There is reluctance of the pharmaceutical company, Abbott Laboratories, and of the FDA to expedite approval of this drug now in use for over a decade by more than 100,000 patients in Europe, Britain and Japan. In addition to its role in facilitation and coordination among the protaganists, NDP has engaged in testing the drug in the U.S. at one of its comprehensive centers and on patients in its own section of our intramural program here at NIH. Surely these are examples of fundamental contributions to technology transfer.

The five comprehensive epilepsy programs supported by NDP in Virginia, Minnesota, Washington, Georgia and Oregon continue that theme. Each of these programs are approaching their first major review of their progress and decision with regard to continuation. As would be expected, there are differences. One center has been most productive in clinical research; another has excelled in development of patient (outreach) services; and a third has done especially well in technology transfers. Of those in their third year each of the three centers has achieved 90 percent of goals set three years ago. Without significant increases in NINCDS appropriations, full implementation of these comprehensive epilepsy programs must be further delayed, since the $1-1.5 million budgetted for that purpose has simply not been available.

STROKE AND TRAUMA

The Stroke and Central Nervous System Trauma Extramural Research Program (STP) is responsible for research on two of the most important public health problems in this country, namely, stroke (the third leading cause of death and one of the leading causes of severe, chronic disability) and CNS trauma (the significant factor in the fourth leading cause of death, and the major crippler of young adults). Of the total research investments by the STP, some 45 percent is devoted to stroke, 34 percent to trauma (about equally divided between head and spinal cord injuries), and about 16% for central (especially spinal cord) regeneration. As already noted, the $25 million STP budget is a pitifully small fraction of the costs of these disorders to the U.S.--costs which annually amount to many billions of dollars, and the STP budget can hardly compare with those available to NHLBI and NCI for research on the number one and number two causes of death in the U.S. Such disparities in Congressional and NIH budget allocation priorities are increasingly difficult for us in NINCDS to disregard or to explain to the community.

In stroke research there is much activity. Two studies have now been reported on the effects of chronic aspirin therapy on transient ischemic attacks (TIA's or "little strokes") and subsequent occurrence of stroke. The Canadian study in particular indicates significant prevention in patients with multiple TIA's but found sex differences (little benefit for women) and a negative correlation with hypertension. Thus, we are confronted here with some new questions and future challenges. The STP is currently supporting an epidemiological study on TIA's in a stable older adult population, so that we can better understand the characteristics and significance of these attacks. The use of the Doppler ultrasound scanning technique is proving to be highly applicable for identifying cases of carotid artery stenosis or occlusion. STP has just embarked on a 3-5 year, $5 million prospective evaluation, at 20-30 centers, of the efficacy of the extracerebral-to-intracerebral (EC-IC or superficial temporal-middle cerebral) artery anastomosis for prevention of further TIA's or subsequent stroke. Relevant new experimental studies include the use of infrared scanning of the brain to evaluate the oxidation of state of brain tissue cytochromes, as a measure of tissue oxygenation, and the isolation and study of brain capillary vessels for their enzyme and transport properties.

These latter techniques are also applicable to trauma problems as well. In addition the newly developed techniques of emission-isotope computerized brain scans (analogous to the CT scans with X-rays) make it possible to directly query tissue perfusion and metabolism. We think this may prove particularly helpful in evaluating stroke and trauma cases, notably those in coma. STP-supported studies indicate the feasibility of utilizing prognostic profiles to improve our handling of the comatose patient. With these techniques, plus more intensive approaches to the edema problem, including a controlled clinical trial now in the planning stage for evaluation of high doses of steroids, we may reasonably expect the momentum in stroke and trauma research to increase significantly.

As a result of the recent interest in central regeneration, research in this area has been stimulated by STP staff and workshops. The STP investment in regeneration research has increased manifold. After considerable delay we have obtained the authentic Soviet enzymes used in the Matinian experiments purporting to show enzyme-induced regeneration of spinal cord in cord-transected rats. Contract studies are now under way designed to exactly repeat the Soviet experiments. Several recent studies add to the potential promise in the regeneration area. These involve the use of donor segments of peripheral nerve grafted into other nerves or into spinal cord to facilitate axonal regeneration across the transection gap. Particularly exciting are the observations that with appropriate immunosuppression techniques, the donor nerve graft can be successfully used from other animals of the same species or from other species including man. The characteristics inherent in the Schwann or sheath cells of the graft can be preserved. This suggests a model system for studying human neuropathies (including diabetic neuropathy). Such studies (part of the responsibility of our NDP group) are being promoted for the coming year.

A particularly important initiative undertaken by the STP during this year has been the funding of the feasibility stages of both comprehensive CNS trauma and comprehensive stroke programs. These are designed to address the difficult problems of evaluation of research findings in terms of community practice and patient prognosis, of epidemiological aspects of stroke and trauma in community settings (sources of accidents, handling of patients in the health care system, what goes on in nursing homes), and of applied clinical research, particularly how to modify research center technology to be useful in the community context. We believe that the current trend of decreasing mortality from stroke and CNS trauma reflect prompter and better management. We lack the answers to two obvious questions. Are those who are being salvaged or kept alive useful survivors? And can we improve the salvage rate by the kinds of research-community interfaces contemplated for these comprehensive stroke and comprehensive CNS trauma centers?

FUNDAMENTAL (BASIC) RESEARCH

The final extramural program, the Fundamental Neurosciences Extramural Research Program (FNP), not only funds basic neuroscience research of no immediately obvious clinical relevance or of a cross-cutting general nature but is also responsible for monitoring the basic research funded by the other three extramural programs and the intramural program of the NINCDS. This is a large responsibility since by several different types of analyses during this year at least 55 percent (and perhaps as much as 70 percent) of the total NINCDS research portfolio can be classified as basic research. This should not come as a surprise in view of the inherent complexity of the central nervous system and in view of the fact that for most of the neurological and communicative disorders the causes are still unknown, preventive and specific diagnostic measures are still lacking, and there are few definitive therapies or cures. Thus acquisition of new knowledge by basic research remains our number one priority.

INTRAMURAL RESEARCH

The NINCDS Intramural Research Program (IRP) is smaller than the four extramural programs but the basic and clinical investigations in its laboratories are being carried out by a particularly dedicated and gifted group of scientists. One of the most appealing aspects of their work is the extent of collaborative studies among the various IRP units and with other NIH and extramural groups. Let me cite a few examples of their current contributions.

With the use of highly purified specific antibodies to myelin basic protein, coupled with a peroxidase staining method, oligodendroglia and their processes enfolding axons can be demonstrated long before their visualization by traditional techniques. One fascinating finding by this technique is the interaction of process from one oligo cell with as many as 7-10 axons, often from several different populations of axons, so that one oligo can encompass an extraordinarily complex field of influence. This technique should prove especially useful in studying myelin "plasticity" which accompanys the metamorphosis of tadpole to frog. Studies on the encephalitogenic component of myelin basic protein indicates that the 19-amino-acid sequence from guinea pig myelin is 100-fold more potent than the same sequence from rat which differs only by the substitution of a single amino acid (a threonine for a serine residue). Such specificity must have some very broad implications.

Continued studies of the nature of the muscle receptor block in myasthenia gravis reveal that there is a cholinergic receptor on thymic epithelial cells. Thus the question arises: is it this cell which produces receptor antibody that cross-reacts with the receptor at the neuromuscular junction? If so, thymectomy, often effective in myasthenia, would assume greater importance, and the newly developed gallium scans to locate thymus tissue and improved surgical approaches to the gland will prove most helpful.

Our understanding of motor activity is being enriched by data from free ranging animals with chronically implanted electrodes recording the afferent (incoming) input to the spinal cord. The records indicate a tremendous informational input from muscle spindles, cutaneous receptors, joint capsules, etc. Addition of recordings from motor units (muscles) will be attempted later. In these kinesthesiological observations such preparations will be immensely useful for the study of the effects of perturbations in the system.

Enzyme replacement therapy for Gaucher's disease pioneered by our intramural scientists has now been repeated and confirmed by another U.S. group and also by a British group. Large scale commercial production of the enzyme is now ongoing. The possibility of producing an animal model of Gaucher's disease is under active investigation. Such a model would be invaluable for detailed evaluation of enzyme replacement therapy. Other experimental animal studies have already demonstrated the feasibility of utilizing techniques for temporary opening of the blood-brain barrier to deliver systemically-administered enzymes into the central nervous system.

Another type of study has demonstrated the transfer of experimental allergic encephalomyelitis (EAE), an animal counterpart of multiple sclerosis, from one genetically identical animal to another by injection into the recipient of a subfraction of lymphocytes isolated from the donor animal's lymph nodes. Moreover in EAE animals during the quiescent phase, T-lymphocytes from spleen (but not lymph nodes) can be reactivated by culturing them in vitro with Concanavallin A, so that when they are injected into a recipient animal the disease is produced. Since it is known that Concanavallin A alters the surface characteristics of cells, this experiment suggests analogies to triggering events which presumably precede attacks of myasthenia, MS or Guillain-Barré syndrome.

A special study of twins in whom at least one twin has developed MS indicates that the incidence of MS in twins may be 150-fold higher than that in the normal population and 10-fold higher than that observed in the Shetland and Orkney Islands, the MS focus with the highest known incidence. In those twin members clinically free of MS, there is evidence in their CSF for the characteristic oligoclonal bands of IgG. Such twin members are being followed to answer the question of whether this finding presages later onset of MS. In addition the oligoclonal IgG bands may prove useful in distinguishing cases of simple optic neuritis from cases of MS presenting initially with ophthalmic symptomatology.

The studies of defective infectious (DI) viral particles continue to be of great theoretical and practical interest. These DI particles contain only part of the viral genome complement, but they carry the same protein coat as the normal virus and hence can stimulate antibody production and the production of the endogenous antiviral agent, interferon, by host cells. One of the DI particle strains in use by IRP scientists has proved to be the most potent known producer of interferon and the procedure has been patented and is being developed for large-scale production under contract with a University of Connecticut laboratory. It is most interesting to see the difference in host animal response to a normal VSV virus, which produces an acutely fatal encephalitis, and the response to VSV-DI particles which in the same species produce a chronic paraplegia. Clinically one could not guess that the two neurological disorders are caused by the same agent, modified by the immunological circumstances.

There is increasing interest and concern over the cytomegalovirus (CMV) as a major cause of postnatal cerebral palsy. With development of sensitive specific tests for CMV it has been demonstrated that 3 percent of all pregnant women excrete the virus (and the percentage rises to 8 among lower socio-economic groups). Moreover, one percent of all newborn babies are excreting CMV at the time of birth, thus signifying infection in utero, and up to 10 percent of these babies exhibit some abnormality (ranging from full-blown CMV-inclusion disease in 1: 20,000 pregnancies to the more frequent softer signs like mental retardation, high-tone deafness etc.). Needs for further work, particularly on vaccine development, are obvious.

Despite intensive work throughout this year, progress has been slow in the isolation, purification and characterization of the scrapie-kuru-Creutzfeldt-Jakob atypical slow virus agent(s). Most of the attention has been devoted to scrapie since it is easier to manipulate. There is now preliminary evidence to suggest that when mouse scrapie is added to cultures of mouse neuroblastoma cells, the phenomenon of cell fusion is induced, a finding reminiscent of the multinucleate neurons seen in electron micrographs of human and primate brains a few weeks after intracerebral inoculation with kuru or Creutzfeldt-Jakob infected material. If these observations hold up, it is possible that a screening procedure for these agents may have been found that would reduce the need for intracerebral inoculation, whole-animal screening.

Much current attention is focussed on transmitter agents and on transmission mechanisms. At the synapse, sophisticated combinations of freeze-fracture and electron microscopic techniques have shown that about 50 calcium ions enter the nerve ending per nerve inpulse and that only about 50 micromoles of calcium ions are required to trigger transmitter release from synaptic vesicles. One quantum of transmitter is present per vesicle. The release and recovery of the synaptic vesicles themselves can now be described as a three-stage process. Postsynaptically there is evidence for preformed receptors which are delivered continually to the postsynaptic receptor area for maturation. In the course of these studies evidence has been obtained that developing sympathetic ganglia cells can be manipulated to form either cholinergic or adrenergic synapses, but only the latter tend to persist. These studies suggest separate factors for recognition and for maintenance in synaptogenesis. At the cochlear hair cell and in the cochlear nucleus, circumstantial evidence is strong for aspartic acid and perhaps also glutamic acid as transmitters. This is a significant advance in a previously unexplored area. Studies with specific blocking agents, such as kainic acid, and with deaf mutant animals are in progress to further evaluate the observations. It is especially interesting that studies with the newer anti-Parkinson drugs of the ergot group have confirmed previous suspicions that there are several types of dopamine receptors, one coupled with adenylate cyclase and another not so coupled. The latter appears to be more important for motor disorders. Thus, there may be a whole series of dopamine receptors, with all that that implies physiologically and therapeutically.

A particularly exciting development in the transmitter field has been the recognition of a growing number of peptides as potential transmitters, especially the so-called endogenous opiates, the enkephalins and endorphins. Much attention has been given to their roles in drug addiction and pain phenomena. NINCDS intramural scientists have examined the role of these peptides in cell-to-cell communication, utilizing a culture preparation of mouse spinal cord neurons. According to these investigators, the various peptides do not behave so much like true transmitters as like neurohormones, which increase resolution of the communications process by providing another mode of communication, with pre- and post-synaptic effects at both short and long range distances. In effect these are the body's own "drugs", hence their similarity to pharmacological agents. It is remarkable how complex the picture of central nervous system transmitters and cell-to-cell communication has become. The possibilities for modulation (or for increasing resolution) continue to increase. Considering the extraordinary versatility of the central nervous system, such findings should not surprise us, but they challenge us to try to synthesize the wealth of current information into functionally understandable terms.

SPECIAL ACTIVITIES: WHO PROGRAMS

Before concluding this report I should take note of several special activities involving the NINCDS during FY 1977. As one of the World Health Organization's Collaborating Centers for Research and Training in the Neurosciences, the NINCDS through several key staff members has devoted considerable effort to attempts to develop programs for developing countries. As a temporary WHO advisor I surveyed potential additional centers in Egypt, Kenya, India and the Philippines, plus consultations at the Eastern Mediterranean, Southeast Asia and Western Pacific Regional offices of WHO. Later key NINCDS staff met with staff from the other WHO Collaborating Centers for a consultation and study group session at Abidjan, Côte d' Ivoire, in western equatorial Africa. There plans were developed for initiatives in developing countries of Africa, Asia and South America for stroke, epilepsy, parasitic diseases of the nervous system and malnutrition. These initiatives depend on a modest ($100-200 thousand) funding by WHO. Despite a special trip to WHO headquarters in Geneva (at their request), we have been unable to pursuade them to commit such funds. One initiative is underway, namely the NINCDS-sponsored $100,000 program for fellowships to appropriate professionals in developing countries. Announcements prepared, in collaboration with the Fogarty International Center (FIC), were distributed through WHO regional offices during the summer. Nominations are now being received at WHO Geneva, for review and selection by the Collaborating Center Directors in April 1978, with the first fellows expected at Bethesda by July 1978. The FIC will handle administrative details, while the NINCDS will take care of planning each training program.

GUILLAIN-BARRÉ SYNDROME

Early in FY 1977 the NINCDS was called in to provide expert help with the Guillain-Barré Syndrome problem that surfaced in association with the nationwide swine flu vaccination program. Consultations with and advice to the Asst. Secretary for Health, DHEW, and to the CDC were provided, and a small task force of our intramural scientists and selected NINCDS external advisors was formed to deal with the longer-range aspects of the problem. One IRP branch studied a group of cases in nearby Virginia; a workshop is being planned; and Guillain-Barré is being included in our extramural initiative on the peripheral neuropathies. It is a tribute to our intramural and extramural program scientists how effectively the NINCDS was able to respond to this urgent and very confused national problem.

WORKSHOPS

Several important new workshops were held during the year. In collaboration with Georgetown University Medical School, the NINCDS put on an International Neuroepidemiology Workshop, the first of its type any where and very successful. The proceedings are now in press. NINCDS, NIA and NIMH combined to support an international workshop on Alzheimer's Disease-Senile Dementia and Related Disorders. This workshop took cognizance of the public health importance of the Alzheimer dementia group of disorders and the dearth of ongoing relevant research. Actually this was the first of two planned workshops--this one dealing with research aspects, the other to deal with behavioral and patient care aspects. Again the proceedings of the first one are now in press. There have been two workshops in the epilepsy area. One concerned the Reliable Quantification of Anti-epileptic Drugs. As a result of this symposium-workshop many service laboratories were encouraged to join the voluntary quality control program sponsored by the Epilepsy Foundation of American and funded by the Office of Developmental Disabilities, DHEW. There are now 471 laboratories participating in this voluntary program. The other workshop was convened to develop appropriate chapters for a monograph on the mechanism of action of anticonvulsant drugs. In our experience workshops such as these provide an excellent means of education and technology transfer. They are constantly being supplemented by the regular informational activities of the Institute. One such activity that we are watching closely is a collaborative effort by CDP staff and advisors with NLM to develop an adaptation of the MEDLARS-MEDLINE system to the needs of the communicative disorders. A thesaurus of terms and some additional key journals have been agreed upon, so that the project should become operational next year.

COMMISSIONS

The two Congressionally mandated commissions, on epilepsy and on Huntington's disease (HD), were in full operation during this year and officially terminated at the end of the fiscal year, with submission of their respective reports. Each held a series of public hearings around the U.S. during the year and each utilized numerous task forces and special studies. The NINCDS was responsible for providing staff, space and funds including 6 FTP slots and close to $1.5 million--all out of operating resources, since no special resources were authorized. The report of the HD Commission contains 23 recommendations, the majority of which are directed to NINCDS. The report of the Epilepsy Commission contains well over 400 recommendations, only about 67 of which refer to NINCDS. Together they recommend about $35 million in additional research and activities for NINCDS.

Development of impact analyses of these reports is currently under way by our Office of Program Planning and Evaluation (OPPE). This office is now staffed reasonably well and has during FY 1977 demonstrated its essential role in budget and program planning and analyses. Even though the OPPE still awaits its computerized information system (now in the testing stage) the group has been invaluable in helping all of us meet the many demands placed upon the NINCDS during the year.

The NINCDS has now been subject to the scrutiny of four Commissions (MS, diabetes, epilepsy and HD). In the reports of each one there have been specific recommendations for additional funding both for specific disease-relevant research (and training) as well as for general basic research. For the latter alone the MS, epilepsy and HD commissions recommended additional annual appropriations of $38 million. It should be obvious from the 1974-1977 budgetary history of this Institute that no real increases have been provided by Executive or Congressional budgets to cover any of the recommendations by these commissions. Indeed the NINCDS has been instructed in Congressional committee reports to act upon commission recommendations, and the Institute has done its best to comply. But it must be obvious that in doing so, in the face of not even cost-of-living increases in its budget, the NINCDS is being pressured to alter research priorities on the basis of well-intentioned but nevertheless partisan groups with only partial understanding of the overall mission and program balance for which the NINCDS is responsible. It is particulary disturbing to have groups like the diabetes advisory and coordinating groups dictating to us levels of expenditures for diabetes-related research without regard to other program priorities with which we must deal and especially without regard to our actual funding limitations. I cannot believe that it was the intent either of the DHEW or the Congress to delegate budgetary and programmatic decisions to commissions or their progeny, and yet that is precisely what is happening in the area of diabetes. This Institute views such developments most seriously.

LONG-RANGE RESEARCH STRATEGIES

In FY 1978, with the NINCDS OPPE as a focal point, all of us will face a very special challenge. With concurrence of the NANCDS Council the Institute has undertaken the development of long-range (10-year) research strategies for the NINCDS. Supervised by an NANCDS 4-man Council Subcommittee, serviced by our OPPE, and developed by 7 panels covering the major neurological and communicative disorders, this undertaking is designed to accomplish three major purposes: (1) to establish the current state of the art and to define the current extent of the various neurological and communicative disorder problems.; (2) to identify the gaps in our present research coverage, to single out promising breakthrough areas, and to develop relative priorities for the allocation of resources; and (3) to specify the resources in terms of research grant and contract funds and in terms of manpower needed to accomplish the goals set forth in the second purpose, and also to indicate clearly the consequences in terms of delayed results and greater societal costs of failing to accomplish recommended strategies and timing.

Near the beginning of this report I referred to the chronic underfunding of the NINCDS and to the vast discrepancies between current socioeconomic costs of neurological and communicative disorders and the research funds being expended on them. It is these situations that have prompted us to undertake the planning of long-term research strategies. Input from all sectors of our biomedical and lay constituencies is being solicited. The reports of the 7 panels, plus other groups such as basic neuroscience and neuroepidemiology task forces, will be synthesized and highlighted by our Council subcommittee and our OPPE into a document for general distribution and consumption. We expect it to dramatize our story and our needs better than has been the case in the past. There are serious needs in research training, especially for clinical researchers; there is a desperate need for stability in the research community not only to undergird the research effort but to attract new minds and new ideas; and there is an acute need to provide a critical mass of researchers in order to facilitate the continuing acquisition of new knowledge so essential to the solutions of neurological and communicative disorder problems. If we can document and dramatize these matters for the OD-NIH for the Congress and for the general public (who must back us), then the effort being put into the development of long-range research strategies for the NINCDS will be eminently worthwhile. It is a long time since the NINCDS has looked in broad, long-term perspectives at its mission and programs. Now we are truly challenged to do so.

ANNUAL REPORT
For Period July 1, 1976 through September 30, 1977
Office of Program Planning and Evaluation
Office of the Director
National Institute of Neurological and
Communicative Disorders and Stroke

The Office of Program Planning and Evaluation, as staff advisor to the Director and the Institute on program development and analysis, assists the Director and the Institute program managers in the analysis and evaluation of their programs, and in the development of strategic and operational program plans to meet the long-range goals and immediate objectives of the Institute. The Office provides staff support to facilitate the integration of the program planning, analysis, and evaluation efforts in the categorical program areas and provides the Director and the Executive staff with assistance in setting and articulating the goals of the Institute programs and strategies for meeting those goals. The Office has developed with the staffs of program areas of the Institute, a process to prepare annual implementation plans which form the basis for resource allocation decisions and a development of budget requests for future years.

The Office has, during the past year, begun to develop an integrated Program Information System for the Institute. This will combine scientific program data with fiscal and management data into an integrated Institute-wide data base which will provide necessary information required to effectively and efficiently plan and manage program activities at all levels of the Institute. A review and analysis of Institute program and management information needs has been carried out. Further, an analysis of available information data systems both within and outside of the Institute is also under way.

The Office has been a focus within the Office of the Director, NINCDS, and of assistance to all parts of the Institute, in (a) gathering and coordinating input for, and in developing special reports (oral or written) and issue papers concerning the Institute's program efforts in specific areas of special interest to NIH. These include support of clinical application, control/demonstration, technology assessment; participation with the Commission and Coordinating Committee on Genetics; (b) in reporting on interagency concerns such as NINCDS studies in heart/lung, cancer, diabetes, nutrition, and arthritis; (c) in working closely with all Institute elements in developing a number of major planning and budget documents including the Forward Plan, projecting the Institute's objectives and resource needs through Fiscal Year '83, and the preliminary Fiscal Year '79 budget for OMB. The Office developed the Evaluation Plan for both Fiscal Years '77 and '78, and is carrying out some of the ongoing evaluation projects and monitors the management of the others.

The Office has developed, within the Institute, an implementation planning process which offers an opportunity to all programs to provide comparable input about program needs, opportunities, priorities, etc., in a uniform fashion for use in resource allocation decisions by the Director. On the basis of these submissions, discussions and negotiations, OPPE develops an Institute operational plan which documents program plan agreements and resource allocation agreements to carry out the Institute programs for the coming year.

The Office provides a central management focus for the Institute's science information programs, which provide summarized literature review and bibliographic references to the research and clinical community in fields related to the Institute's mission. A plan is being developed for the evaluation of these programs including the analysis of the options, and possible alternative methods of providing for the objectives of these services. It is hoped that this evaluation can, by mid-FY'78, provide to the Director necessary informaton for the decisions concerning the supplementation and/or restructuring of these information services.

The Office, together with a special subcommittee of the Council, has planned and begun upon a strategic planning process which will review the Institute's overall program in the context of current public health needs and research opportunities to provide for establishment of long-range goals and long- and short-range strategies for the Institute.

The Office continued to work on the following research projects:

1. Study of Mental Retardation -- A record anchored retrospective study of mental retardation cases as diagnosed in Georgetown University Clinic with intent to discover etiological associations with selected prenatal variables (genetic, socio-economic, obstetric) as these are compared in index cases with sib and non-sib controls from the Georgetown Center as well as from the COLR Study. (See Annual Report Project Number Z01 NS 01144-15 OPPE.)

2. Public Health Implication Study -- A comparison of death rates (1959-1965, fetal, neonatal, infant mortality): (a) In the COLR populations, intra- and inter-institutionally, annually; (b) In the population of cities where these institutions are located and from which the COLR studies have been selected; and (c) In these two population samples. (See Annual Report Project Number Z01 NS 01146-15 OPPE.)

3. Study of Labor (.01 man years) -- The pediatric outcome of vaginal deliveries in normal pregnancy compared along selected parameters with the outcome of elective Caesarean deliveries, with intent to discover, if possible, the effect of uterine contraction, on time and prematurely, on normal growth and development of the offspring. This study was reactivated during FY'77 in collaboration with NICHHD; analysis is actively under way. An obstetrician in another Institute is interested in collaborating in the analysis and writing of the manuscript because C/S is more and more used in obstetrical practice and, as such, needs careful evaluation. Computer retrieval of data on normal deliveries has been delayed on account of difficulty in coding the "normal pregnancies" in COLR data.

4. A Study of Comparative Health (.01 man years) -- In selected ethnic U.S. groups, a survey of incidence and prevalence of MS, ALS, Parkinson's disease, etc., and other bio-psycho parameters to compare with adequate controls from samples of population of the same ethnic origin in other lands. The comparison will be made in a selected period of time and the analysis will hopefully reveal some insight into the mechanism (genetic or environmental) of production of these selected parameters. Contacts with interested investigators in Lebanon and Israel have been made and data collection shall proceed as soon as proper population samples have been selected here and abroad.

5. Comparative Study of Schools of Thought in Medical Science and Practice (A study in medical care) (.01 man years) -- In the U.S., allopathic medicine is thought and practiced side by side with homeopathic medicine, etc. Many systems of medicine are prevalent in India. In Lebanon, the American University has a faculty of medicine and the French University has another faculty, thereby offering a chance to compare French and American medicine. This has implications not only to the systems of delivery of medical care but also to the growing concern in training physician aides even in the developed countries. Contact with interested collaborators in Lebanon and the Mid East Center in the University of Pennsylvania has been made. Data collection is imminent.

6. Administration Research in Collaboration with NICHHD (.02 man years) -- This is a test of the hypothesis that authority and responsibility in any bureaucratic hierarchy are equal only in middle levels and that authority disproportionately increases the higher the level, and conversely, responsibility disproportionately increases the lower the level. A draft manuscript has been prepared and will be submitted for publication jointly with a political scientist from another Institute.

The Office staff has also been active in a number of associated activities such as coordinating the Combined Federal Campaign, U.S. Bond Drive, the Committee on Science and Human Values, and NINCDS EEO Advisory Committee.

CONTRACT NARRATIVE
Office of Program Planning and Evaluation, NINCDS
Fiscal Year 1977

NINCDS PROGRAM INFORMATION SYSTEM (PINS) (N01-NS-6-2345), JRB Associates,Inc., 8400 Westpark Drive, McLean, Virginia 22101.

Title: Design and Specifications for a NINCDS Program Information System (PINS)

Contractor's Project Director: Laurence C. Novotney

Allocation: $275,840

Objectives: The Program Information System (PINS) is an evaluation project to develop the methodology for, and system design to support the efficient input, storage and retrieval of the scientific and programmatic data required to effectively plan, analyze, evaluate, and manage program activities at all levels of the NINCDS. In addition to assessing the present and future informational needs of the Institute, available information data systems both within and outside of the Institute will be reviewed and analyzed. The PINS will be designed to enable Program Managers to have more effective command and control of their program's information in terms of the scientific substance, as well as the budgetary and administrative data.

Major Findings: The contractor completed interviews with the NINCDS staff to ascertain the Institute's information requirements. After analysis, these requirements were documented in the Functional Description and Requirements Report. This report then constituted the basis for the Conceptual Design for the Program Information System. Five components of the PINS were identified. For the Budget/Program Analysis component of the PINS, NCI's Fiscal Projection Model (FPM) was evaluated to determine its feasibility to satisfy the fiscal reporting and projection techniques portion for the NINCDS. FPM demonstrated its ability to satisfactorily provide these functions. An integral part of the Project/Program Status and Reports component of the PINS is a scientific classification system. Recognizing the inadequacies of the present classification system, Institute staff and the contractor are designing the structure of a new and more descriptive and retrievable Institute classification system which will consolidate information from both Extramural and Intramural Research and provide accurate and timely responses to questions from the Administration, the Congress, or the public. In order to determine the most appropriate data base management system for use with the PINS, the contractor with the support of an independent consultant completed an analysis of commercial data base management systems and submitted his recommendation to the Institute. The program specifications are being developed based upon the approved Conceptual Design and incorporating data processing capabilities compatible with the recommended data base management system.

Significance to the Program of the Institute: As scientific and programmatic information concerning grant, contract and project management, financial (e.g. budget and operating expenses), program projection requirements, and personnel continue to increase in size and complexity, rapid access to pertinent facts on a timely basis is essential for effective management. By utilizing a database management system (DBMS) which provides controlled access to the Institute-wide data file, PINS will meet this need and make data acessible when and where it's needed, and in a form that can readily be used. The DBMS greatly simplifies programming, maintenance and operation of such applications and data thereby providing an improved level of data access and management. In addition, the PINS will demonstrate a practical means of maintaining data currency, validity and compatibility between it and existing NIH systems such as IMPAC, CAS, etc.

Proposed Course: The work on the JRB contract has been favorably received and OPPE would like to initiate the implementation phase of this project. Conceptually the implementation phase would be as follows:

a. Competitive award for the computer programming, debugging and testing.

b. Sole source award to the present contractor to include monitoring the programming product of a. above and for development and implementation of the entire NINCDS Program Information System.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01144-15 OPPE

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

An Instrument for the Conduct of a Retrospective Study of Seizures, Cerebral Palsy, Mental Retardation (M.R.) and Other Neurological and Sensory Disorders of Infancy and Childhood.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Z. A. Shakhashiri, M.D.	Special Assistant	OPPE, NINCDS
OTHER:	Mr. Ernest Harley	Systems Analyst	EBRP, NICHHD

COOPERATING UNITS (if any)

LAB/BRANCH

OD-NINCDS

SECTION

Office of Program Planning and Evaluation

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.02	.02	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS -Record anchored study

SUMMARY OF WORK (200 words or less - underline keywords)

A retrospective instrument has been designed (index cases, with sib and non-sib controls) for record-anchored Study of Mental Retardation (cases selected from Georgetown (G.T.) University Hospital Specialty Clinic) as a pregnancy outcome. Ten hypotheses were being tested, related to the biological and psycho-social mechanisms underlying the occurrence of damage in the growing white child by age 5 years: anoxia, toxic influences on brain, metabolic influences, trauma to the head, infection of brain, dehydration of child, genetic or familial factors, socio-economic status, prematurity, and nutrition. Heart disease, prenatal hypertension, breech presentation, bleeding, Caesarean Section (C.S.) and Cephalopelvic Disproportion (C.P.D.), were noted more frequently in mothers of M.R.; and meconium, B.W.<2500, and neonatal head injury more frequently in M.R. children themselves, than in the corresponding sib and non-sib controls. Findings from G.T. data base are being compared to findings from prospective Collaborative Perinatal Research (COLR) data base, using the same retrospective instrument. In view of less incomplete data in the latter, this comparison would confirm or invalidate the initial findings from the former.

Project No. Z01 NS 01144-15 OPPE

Project Description:

Objectives: Design an instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood in order to test certain basic and important hypotheses concerning the occurrence of neurological damage.

Methods Employed: Recognized damaged outcomes of pregnancy, especially mental retardation, have been studied and related to defined perinatal or postnatal events which are involved in the biological or psycho-sociological mechanism underlying the following hypotheses: (1) anoxia, (2) toxic influences on the brain, (3) metabolic influences, (4) trauma to the head, (5) infection of the brain, (6) dehydration of the child, (7) genetic or familial patterns, (8) socio-economic status, (9) prematurity, and (10) nutrition.

Current Status: Upon review, the manuscript was criticized on the basis of possible bias inherent in incomplete data as presented. Since the data base is available on tape, another extended analysis is under way, comparing as well, the emerging findings from this retrospective study with those from the prospective COLR study when subjected to the same instrument. The validation of findings from the retrospective study would thus be possible.

The retrospective instrument designed for the collection and analysis of the Georgetown Study data has retrieved data from the COLR data base. The comparison of findings from these two sources of data is under way. Its completion during FY'78 shall be made possible based on experience gained during FY'77 in handling problems inherent to comparing data from 2 separate record-anchored studies, initially separately designed and with "different" operational definitions of variables used.

Honors and Awards: None

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01146-15 OPPE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Public Health Implications Study of Perinatal Mortality in the Collaborative Study (COLR) and in the Collaborative Study Cities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Z. A. Shakhashiri, M.D.	Special Assistant	OPPE, NINCDS
OTHER:	Mr. Ernest Harley	Systems Analyst	EBRP, NICHHD

COOPERATING UNITS (if any)

LAB/BRANCH
OD-NINCDS

SECTION
Office of Program Planning and Evaluation

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS -Record anchored study

SUMMARY OF WORK (200 words or less - underline keywords)

Perinatal mortality of COLR children (single births 1959-1969) was compared by institution, race, sex, birth weight (b.w.), gestation length (g.l.). Regional trends appeared in a steady decline, except for 1962. Females, for both races, have lower b.w.; non-whites, for both sexes, have lower b.w., and shorter g.l. Perinatal mortality, as well as fetal mortality, is lower for non-whites at short g.l. and low b.w., and higher at long g.l. and high b.w. These data formed the basis of a term paper by Dr. G. Bartlett, in a Sociology course at Western Reserve.

Similar data are being analyzed from those COLR cities which supplied data upon request. Comparison with COLR data variables (mortality, g.l., b.w.) might appear in a manuscript by the end of FY'78. Additional studies are contemplated for later, on multiple births, controlling on factors known to effect mortality, g.l., b.w. (e.g.) parity, maternal age, prior pregnancies, legitimacy, etc.

Project No. Z01 NS 01146-15 OPPE

Project Description:

Objectives: To evaluate fetal and infant mortality of the Collaborative Study population and of the cities from which that population is drawn, with the aim of comparing the two populations, city by city, and institution by institution, on mortality characteristics.

Methods Employed: In addition to the data previously available from the National Center for Health Statistics for perinatal events, detailed data on natality and perinatal mortality were sought for the study cities from either the state or city health departments, whichever had jurisdiction for these records. The data include figures for livebirths, stillbirths, and deaths under 24 hours, 1 day to 7 days, 8 days to 28 days and 1 month to 12 months, evaluated by birth weight, length of gestation, race and sex, and plurality for the years 1959 through 1966. Corresponding data were compiled by institution for the PRB study population. The state or city health departments furnished either tabulations or raw data to be tabulated.

Considerable effort has been expended to create a usable data file of the external data being obtained in connection with this study. The aim is to provide a file with more general utility than the limited scope of this study. When such a file is created, the information necessary to make use of the file will be made available to interested persons.

Current Status: There has been an unavoidable delay in the preparation of the manuscript describing the comparative trends of fetal, neonatal and infant mortalities in the COLR population and in the population of the cities where COLR institutions are located.

The mortality trends of the COLR population are already in view, but are to be compared with those in the cities where the COLR institutions are located before the manuscript can be considered complete. After discussions with select epidemiologists and statisticians from another Institute, it appeared that the data previously collected on the cities where COLR institutions are located, are not adequately complete, except in one or two cities. A decision is to be made during FY'78, after proper consultation, regarding any further attempt at comparative analysis and/or preparation of a final data file of the external data, to be ready for use by any researcher for any sensible purpose. Dr. Bartlett's term paper on the internal data shall be submitted for publication.

Honors and Awards: None

Publications: None

Office of Scientific and Health Reports (OSHR)

NINCDS information, publications, press, and public affairs activities are centered in the Office of Scientific and Health Reports (OSHR). The office is divided into three sections: Scientific Publications, Health Reports, and Public Inquiries.

The Office is responsible for advising the Director and executive staff on ways to enhance the Institute's public and professional image and on the effective interpretation and reporting of Institute-conducted and supported research findings. These findings are of interest and concern to many audiences, including Congress, the Department and other agencies of government, scientists, physicians, voluntary health agencies, and the general public. The Office initiates many projects, and responds to many Congressional, Departmental, NIH, internal, and public requests.

This year and for the past 21 years, the OSHR has been responsible for producing Special Reports on disease problems designated by the House Subcommittee on Appropriations. These state-of-the-art reviews describe the disorder, present the Institute's program, review research advances of the past year including any new developments in therapy, and briefly project the outlook for the future. Four Special Reports requested this year were: Stroke, Epilepsy, Spinal Cord Injury, and Multiple Sclerosis. The Office also provided sections for insertion in two Special Reports published by other Institutes, on the subjects of genetic diseases, and diabetic neuropathy.

Coordination of the Institute's program with the programs of some 60 private voluntary agencies and professional societies is a major function of the OSHR. This continued in Fiscal Year 1977 with the performance of many information services, including an annual directory of voluntary agencies; preparation of speeches and information materials as requested on specific disorders; meeting with representatives of voluntary agencies to provide advice and to obtain information about their programs; assisting with voluntary agency annual, scientific and press meetings; and various other types of assistance.

Six agencies requesting cooperation requiring considerable time during 1977 were the National Multiple Sclerosis Society, the Muscular Dystrophy Association, the Committee for the Control of Huntington's Disease, the Amyotrophic Lateral Sclerosis Society of America, the Epilepsy Foundation of America, and the Myasthenia Gravis Foundation. A sampling of the types of assistance rendered during the year includes the following: preparing a new brochure and clearing it with the voluntary agency; serving on the journalism awards committee of two associations; helping to set up a special award one of the societies wished to give; reviewing the NINCDS program with one of the Agencies to find possible areas of additional cooperation; taping a TV program for an association; providing a large number of copies of a new publication for a foundation; and writing a speech to be given at a fund raising dinner.

A new concern of the Institute has been better dissemination of information to physicians and paramedical personnel. This year the Office of Scientific and Health Reports inaugurated a service for journals in the fields of neurology, neurosurgery, otolaryngology, speech, and the neurosciences, called NINCDS Notes. This consists of four pages of copy, produced monthly, and includes program and administrative developments in the Institute. The audience is primarily heads of departments, chairmen of training programs, grantees, potential grantees, and clinicians.

OSHR now contributes regularly to an NIH service to the Journal of the American Medical Association, providing items of interest to clinicians for a special section appearing monthly. As of July 15, 1977, five NINCDS items had been contributed to this new service.

The Office has continued its medical education program by way of video tapes, on which are edited versions of medical and scientific lectures given at NIH or by grantees. About 60 of these have been produced, and there has been substantial demand for them by medical schools, teaching hospitals, government hospitals, drug companies, private practice groups, and individual scientists.

Each year the Institute submits 8 to 10 articles with pictures for an NIH brochure entitled NIH Research Advances. Ten articles were submitted in 1977, and work was beginning on the 1978 edition in July, 1977. Fifty thousand copies of the 1977 Research Advances were printed, of which 35,000 were distributed to senior medical and dental students, and all senior grantee investigators.

OSHR is responsible for a variety of other activities. One of these is the processing of requests filed under the Freedom of Information Act. Under new laws, it has been necessary for each Institute to appoint a Freedom of Information Officer and a Privacy Officer. A considerable amount of material, particularly material relating to grants, must now be made available promptly on request. In addition, meetings are held to clarify policy, and reports must be made to NIH and HEW periodically.

In the day to day operation of the OSHR, the three sections produced publications, audiovisual materials, reports, press material, answers to public inquiries, and served as an information resource on the many disease categories within the Institute's purview.

The Office also provides services in connection with special events. The most recent major special event was celebration of the award of the Nobel Prize in Medicine to an Institute scientist, Dr. Carleton Gajdusek, for his work with slow viruses. A press conference was conducted at the NIH Clinical Center; a press kit with photos and background material was provided, and inquiries were answered for many days afterward. Assistance was also provided in executing NIH and Institute tributes to Dr. Gajdusek, including a printed program for an afternoon event in the Clinical Center auditorium, and help with securing a band, flowers, videotaping, and many photographs.

Scientific Publications Section

The Scientific Publications Section produces and distributes publications, both for the general public and for the various scientific and professional audiences. Publications production services are provided to various administrative units of the Institute, *ad hoc* committees preparing reports, and outside organizations in the neurological and communicative disorders fields when sufficient need is demonstrated. The services include planning, writing, editing, design layout, clearance, distribution, storage, and later revising and reprinting according to demand. The Section works with the NIH Printing Unit, the Medical Arts and Photography Branch, and the Government Printing Office. It also serves as the Institute's supply center for publications.

The Institute's *Monograph Series* is being continued. Fifteen publications in this series have been printed to date, and seven of these continue to be distributed by the Section and by GPO. Five new monographs in various stages of production are a set of manpower surveys in the following areas: neurology; speech pathology and audiology; otorhinolaryngology; neurosurgery, and basic and communicative sciences. The most recent in this series, *The Scientific Basis of Spinal Manipulative Therapy* (June 1976) has just been reprinted for sales copies by GPO at $4.75 per copy. About 4,000 have been distributed by the Publications Section, and 1,500 have been sold by GPO.

The Institute's highly popular *Hope Through Research* pamphlets and companion fact sheets on neurological and communicative disorders continue to be revised and reprinted as necessary. This year a new pamphlet on Parkinson's disease was published; a new pamphlet on amyotrophic lateral sclerosis was approved for printing, and a new pamphlet on multiple sclerosis was written and sent forward for artwork. A new pamphlet on backache was written and submitted to scientists for review; and four new fact sheets were in preparation, on Friedreich's ataxia, serum concentrations of anti-epileptic drugs, dysautonomia, and diabetic neuropathy. These publications, three of which are also in Spanish, are widely circulated to private voluntary groups for distribution to patients and health workers and to students for use in health related classes and course work. 350,000 Hope Through Research leaflets are being distributed via pamphlet racks in 220 supermarkets in the Baltimore-Washington area. This distribution program, performed under a contract, will be expanded as printing funds allow. It offers the possibility of placing the pamphlets in view of some 40 million persons per week, nationwide.

The annual special reports to Congress, used as background material for the appropriation hearings, are printed as pamphlets following the hearings, and used as public information materials. This year four were prepared, on spinal cord injury, stroke, epilepsy, and multiple sclerosis.

Numerous other publication or revision projects were completed by the Section. At the close of the fiscal year there were 43 Institute publications being stocked and distributed, and some 15 publications produced by other organizations but stocked in small quantities for use in answering inquiries.

Public Inquiries Section

This section directs the preparation of replies to written inquiries and telephone calls concerning research findings on some 200 neurological and sensory disorders. Many of the inquiries involve difficult subject matter, and before fully factual responses can be written staff of this section must coordinate closely with intramural scientists and grantees. Likewise, policy inquiries require close interaction with the Office of the Director, NINCDS; Institute Program Directors; the NIH Secretariat; and the HEW Secretariat.

However, inquiries addressed to the NINCDS are not confined to research; they also include patient care, rehabilitation, services, and the economics of neurological and sensory disorders. Complete answers to these requests necessitate running down pertinent information from other components of the Department, other agencies of Government, and from State Agencies through which services and financial aid are funneled.

Last year, 1,819 individually prepared responses were sent to the Institute's lay and medical audiences. In addition, 182 responses to controlled letters from the Congress and the White House were written and coordinated with the NIH Secretariat. Many other inquiries were answered with printed materials. In all, NINCDS responded to requests for 445,728 publications.

The responsibility for designing, scheduling, and manning the Institute's Program Exhibit is centered within this section. In Fall 1976, it was shown at the AAOO meeting in Las Vegas, the ASHA meeting in Houston, and the Neurosciences meeting in Toronto. Early in 1977, it was decided that separate exhibits for the neurological and communicative programs would be more effective. Accordingly, a new modular exhibit was constructed to depict the neurological interests of the NINCDS and later was shown at the AAN and ANA meetings in Atlanta and Chicago respectively. Plans for a second modular exhibit for the communicative program are under way, and the exhibit will be completed in time for the Fall meetings on hearing and speech disorders. The modular exhibits are portable and save the Institute many hundreds of dollars each year in shipping, handling, and setting-up charges over the former exhibit.

These showings generate hundreds of requests for information about the Institute's programs. The ASHA meeting is especially valuable for getting NINCDS publications for the lay public into the hands of students and paraprofessionals.

This section provides materials for the Institute's Advisory Council meetings, updates Council Directory, and plans the annual Council dinner. The head of the section also serves as information liaison with the Extramural Activities Program and with the Program Directors in the Federal Building, is responsible for grantee identifying research appropriate for use in Institute reports and publications, and writes annual special reports for Congress on cerebral palsy and spinal cord injury.

The head of the section also serves as the focal point for the Institute in discharging its responsibilities under the Freedom of Information Act.

Under this Act, the Public Inquiries Section responded to 105 requests for summary minutes of the meetings and to 35 requests for substantive information about the Institute's programs. It also provided the Department with annual reports on the Freedom of Information activity.

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Health Reports Section

The Health Reports Section is responsible for preparing and distributing reports about health research (printed, filmed and audio/video taped) to scientists, members of Congress, voluntary agencies and the public for general information and professional medical education.

The Section operated an audiovisual production/taping studio facility in a converted laboratory in the Auburn Building from February 1975 to July 1977. The facility included cameras, switcher, and precision videotape cassette editors and production capability for making presentations of medical lectures by the scientists concerned.

Using those and other facilities, the Section this year produced ten new lectures in the Neuromuscular Disease series, seven lectures given at a June NINCDS International conference on Alzheimer's Disease, four introductory lectures on neuroepidemiology, one on enzyme deficiency diseases and three exhibit presentations about NINCDS and various of its programs.

Since they were introduced for free loan to interested users, in January 1975 the original 28 videotapes on neuromuscular diseases have been requested 2,592 times by 235 users for an average of 93 bookings per tape. There were an average of 163 showings of each tape for a total of 4,577 showings. The average number of viewers per tape was 4,008 with total audience of 112,221 for all tapes.

During the year Section staff members refurbished the Neurological Information networks exhibit and used it for four months as an exhibit about NINCDS on display in the main Building 31 corridor. It incorporated a seven minute videotape about the Institute. The Section refurbished the same exhibit again and exhibited it at the American Academy of Neurology meeting in Atlanta where it incorporated another five-minute videotape production about NINCDS neuroepidemiology programs. The Section designed a new exhibit for neuroepidemiology which can be carried to meetings thus greatly reducing shipping expenses and set-up and break-down time and inconvenience. (This one was exhibited at Chicago meeting of the American Neurological Association). An agreement was negotiated with the National Medical Audiovisual Center (NMAC) in Atlanta to distribute thirty-two additional video taped neurological presentations produced over the last two years. A contract with Association Sterling Films under which the first twenty-eight such tapes were distributed will be closed out when there are no further requests for them. The Section shot a great deal of video tape footage in and around Bethesda and the District of Columbia for use in the neuroepidemiology productions and for future use as stock footage in NIH video productions. The staff provided much assistance to the National Heart and Lung Institute in their production of an orientation film and assisted Institute offices and other NIH agencies with their audiovisual projects.

Annual Report
for Period July 1, 1976 through September 30, 1977
Office of Biometry and Epidemiology
Office of the Director
National Institute of Neurological and Communicative
Disorders and Stroke

The Office of Biometry and Epidemiology (OBE) is responsible for the development and implementation of research programs in biometry, epidemiology, and systems analysis to advance medical knowledge and reduce the impact on society of neurological and communicative disorders.

This goal can be achieved by means of information obtained from three broad categories of research studies. They are:

1. Broad, descriptive population studies.
2. Analytic epidemiologic studies.
3. Experimental studies.

Priorities have been established for the design and imple- ntation of studies in all three categories, with the highest iorities established in the order listed. In part, studies pend for their development on those from antecedent categories.

Broad descriptive studies include the Head and Spinal Cord njury Survey and the Intracranial Neoplasm Survey, both of hich have now been completed. Initial review of these reports uggests that both spinal cord injuries and metastatic tumors f the brain are more prevalent than previously expected. The ata will be published in supplements to the Journals of eurosurgery and Neurology respectively, and these are now n preparation.

Two additional surveys, those for Multiple Sclerosis and troke, are well under way. A feasibility study has been initiated for a national survey of Epilepsy, as well as for a program of periodic surveys of a comprehensive list of neurological and other diseases, in conjunction with the Hospital Discharge Survey of the National Center for Health Statistics (NCHS). This study is a cooperative research effort with NCHS and seven other NIH Institutes to provide annual trend data for disease statistics.

A convulsive disorder questionnaire has been developed for possible use in the NCHS 1979 Health Interview Survey, and is under present review by staff in that organization. This information will be most helpful in supplementing data expected to be developed in the National Epilepsy Survey.

The pilot study for a Survey of Major Neurological Disorders in a county in Mississippi will be accomplished this fiscal year. The survey will provide data on the prevalence of six major neurological disorders among southern blacks and whites.

Analytic epidemiologic studies will be developed from disease-specific repositories of patient data. A computer data base system, which is now in the planning stage, will permit the collection of medical information from standardized forms on large numbers of patients registered in medical centers. The computer system will be applicable to any disease of interest. For example, it will be possible to measure the relative importance of selected patient and disease factors in determining prognosis for the individual patient.

The system will first be applied to patients with stroke, preferably by establishing a data base system for the Comprehensive Stroke Centers. If the system is successful it will next be considered for application to patients with head trauma.

Experimental epidemiologic studies. Discussions are under way with the staff of the Communicative Disorders and Stroke and Trauma Programs for collaboration between these Programs and OBE in the area of clinical trials. If negotiations are successful, OBE may act as a data coordinating and biostatistical center for a number of future clinical trials, such as tests of the effectiveness of the tonsillectomy procedure, effect of heavy doses of steriods on spinal cord injury cases, etc.

Several major epidemiological research contract studies will be completed during this fiscal year, the most important of which is the Study of Multiple Sclerosis in the Shetland and Orkney Islands and Caithness. Several manuscripts have been received from the principal investigator, and several more are in preparation. Perhaps the most significant finding that will stem from this research in this very comprehensive study of a region with the highest reported prevalence of multiple sclerosis in the world is that heredity is not found to be an important etiologic factor in multiple sclerosis.

A major international conference on neurological epidemiology was held in Washington, D.C. Six of the papers were presented by members of OBE, and the Section on Neuroepidemiology held a one-day training session on that topic in conjunction with the conference. The reports will be published in a book entitled "Neurological Epidemiology".

A number of epidemiological investigations in neurology are in various stages of development and implementation. Briefly, they include: a descriptive epidemiologic study of dementia, a study of the association between myasthenia gravis and multiple sclerosis, a study of cardiac disease and/or hypertension as risk factors for subsequent stroke or TIA, a case-control study of Alzheimer's Disease, the development of system and protocols for an ALS Registry and a Registry for Creutzfeldt-Jakob Disease, analysis of U.S. and international mortality data for neurological disease (including Huntington's Disease in particular), a study of life expectancies in neurological diseases, studies of space/time clusters in neurological disease, and the development of protocols and strategies for studying neurological diseases in developing countries.

Brain tumor research includes studies of racial differences in the occurrence of brain tumors, extracranial malignancies metastasizing to a primary intracranial neoplasm, and descriptive epidemiological studies of primary intracranial neoplasms and their patterns, and analysis of discrepancies in their incidence.

Cerebrovascular studies include the measurement of the incidence rate for this disease in children, unusual patterns of the disease, and the incidence during pregnancy and the post partum period.

OBE is preparing a series of disease definitions and classifications. These disease algorithms will be used in surveys and in other epidemiologic studies. Their eventual publication in a disease algorithm monograph will support the design of studies with standardized disease definitions and classifications.

The Section on Epidemiology is also involved in training and education in neurological epidemiology, in the planning of international cooperative studies and symposia, in the preparation of lectures and reviews and in the development of appropriate exhibits. For example, a textbook on the principles of neuroepidemiology will be published in Advances In Neurology. Videotapes on this subject were developed and will be distributed as training films.

In biometry, the Section on Mathematical Statistics collaborated on a broad range of investigations in the Intramural and other Program areas. The most significant of these studies involve the analysis of the data of the Collaborative Perinatal Program, including the Febrile Seizures Study, the Convulsive Disorders Study, the Cerebral Palsy Study, the Neonatal Seizure Study, the Study of Minimal Brain Dysfunction (MBD), the Study of the Effect on Pregnancy of Maternal Seizures, and the Effect of Maternal Infection During Pregnancy on Hearing Defects at Age Seven. The Febrile Seizure Study, for example, has measured the degree of association between early febrile seizures and subsequent non-febrile seizures, while the Minimal Brain Dysfunction Study has developed new scales for the measurement of manifestations in MBD children.

Intramural research consultation in biometry includes studies on the effects of combination chemotherapy in Grade III and Grade IV gliomas, a clinical trial of local 8-Azaguanine in combination therapy for malignant gliomas of the brain, evoked response of the autonomic nerve in the cat, spinal cord arteriovenous malformations-reduction and analysis of AVM variables, glucose utilization in the brain of thiamine deficient mice, image reconstruction theory, study of acupuncture in the treatment of migraine, bromocriptine in Parkinsonism, clinical ictal patterns in epileptic patients, an acoustic study of speech in Parkinsonism, chimpanzee serology study, abnormalities in cellular immunity in ALS and PD on Guam, study of measurement data in man, familial multiple sclerosis: clinical histocompatibility and viral serologic studies, analysis of data from primate maternal nutrition study - Phase II, clinical infection study, abnormal/normal control serology study, and the recovery of usuable data from corrupted EMI scanner tapes.

Mathematically focussed studies include the development of a mathematical algorithm for the design of a morbidity index to permit comparison of the impact of various disorders - particularly chronic, disabling diseases, and a major review and evaluation, submitted for publication, of the currently available methods of spike-train data analyses. In addition, several optimal statistical tests for validating the use of these methods were developed. The mathematical relationship between sensory discriminability and reporting bias in response to experimental pain was studied and the findings will be submitted for publication. A method was developed for the systematic statistical analysis of multiple antibody readings in matched control studies.

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CONTRACT NARRATIVE
Office of Biometry and Epidemiology OBE, NINCDS
Fiscal Year 1977

MASSACHUSETTS GENERAL HOSPITAL (N01-NS-4-2321)

Title: Multiple Sclerosis in the Shetland and Orkney Islands and Caithness, Scotland

Contractor's Project Director: Dr. Raymond D. Adams

Contractor's Co-director: Dr. David C. Poskanzer

Current Annual Level: $120,834

Objective: An epidemiologic, virologic, and immunologic study of multiple sclerosis was undertaken in the Orkney and Shetland Islands, Scotland, where the rates of the disease are 309 per 100,000 and 184 per 100,000, respectively, as compared with the estimated prevalence rate of 40 per 100,000 in Boston. A search for exogenous etiologic factors and factors which might implicate heredity was undertaken.

All patients with multiple sclerosis in the Shetland and Orkney Islands have been identified, and appropriate controls were selected for each patient. For such individuals (both patients and controls), as well as certain family members of these individuals, previous history of infection (to be confirmed by serology), dietary history, sanitation history, history of exposure to animals, occupational history, travel history, and history of allergic diatheses were obtained, and family pedigrees were traced to 1776. Blood samples were obtained from patients, their two age and sex-matched controls, and family members for (1) the histocompatibility determinants HLA, MLC, and B-cell alloantigen Ag7a, (2) blood group typing, red cell enzymes and serum proteins, and (3) viral antibody titers (rubeola, rubella, mumps, varicella, cytomegalovirus, herpes 1 and 2, Coxsackie B3 and B4, parainfluenza 1, 2, and 3, poliomyelitis 1, 2, and 3, echo 4 and 9, and EB virus). The blood samples were shipped to appropriate laboratories for study.

The establishment of family pedigrees and determination of blood groups, as well as their analysis, was sub-contracted (see contract N01-NS-6-2337). With this exception, the results obtained are being analyzed and publication is anticipated in the fall of 1977. The principal investigators of contract N01-NS-4-2321 and contract N01-NS-6-2337 are cooperating in interpretation and publication of results.

Major Findings: Findings determined to date were reported during The American Academy of Neurology meetings in April 1977 as follows:

(1) "A bimodal age at onset curve was observed for Orkney Islands patients with two distinct peaks at ages 20 to 25 and 35 to 40. From this observation it became apparent that there is a subgroup of patients who share the following characteristics: sex, early age at onset, temporal course of exacerbating-remitting disease, and the occurrence of HLA-B7. This group of patients, referred to as Multiple Sclerosis Type I, can be quite clearly distinguished from other forms of multiple sclerosis and may account for the disparities and variations in tissue typing present when the disease is perceived as if it were homogeneous, rather than at least two clinical, epidemiologic, laboratory, and possibly etiologic entities."

(2) "The absence of optic neuritis as an isolated entity without subsequent evidence of other lesions of the nervous system in these islands is remarkable."

(3) "Female Orkney patients who are HLA-B7 positive appear to have a lower titer response to several of the viruses studied, especially measles, as compared to female controls with HLA-B7 and other Orkney patients without this specificity."

(4) "No virus of those studied is clearly associated with multiple sclerosis, either by antibody titer or the presence or absence of previous exposure by history to each virus."

Significance to the NINCDS Program and Biomedical Research: To date research of the etiology of multiple sclerosis follows a divergent path; the quests for environmental and genetic factors continue at comparable rates. Study of genetic and environmental factors in a defined population with the world's highest prevalence of multiple sclerosis will provide directives for future research, justifying emphasis on the pursuit of either genetic or environmental factors.

Proposed Course of the Project: The contract expires on October 15, 1977. The publications resulting from this study are in the final stages of writing and their publication is anticipated this fall.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology OBE, NINCDS
Fiscal Year 1977

THE UNIVERSITY OF NEWCASTLE UPON TYNE (N01-NS-6-2337)

Title: Genetic Study of Multiple Sclerosis in the Orkney and Shetland Islands

Contractor's Project Director: Dr. D. F. Roberts

Current Annual Level: None from the 1977 budget. A sum of $39,179 was alloted to this contract out of the 1976 budget. To date, Dr. Roberts received $19,654.82 of this money.

Objective: (See contract N01-NS-4-2321 for an introductory statement)

Family pedigrees of multiple sclerosis patients and their controls were established for Shetland and Orkney. Blood groups, red cell enzymes and serum proteins on eleven specimens of blood obtained during the March 1976 field trip to the Orkney Island were determined. The pedigree as well as all serology data for Orkney were analyzed in a study of the genetic aspects of multiple sclerosis among the inhabitants of Orkney Island. A similar analysis of Shetland data is underway.

Major Findings: Three manuscripts in various stages of completion are currently being prepared:

1) "Genetic Analysis of Multiple Sclerosis in Orkney"

2) "Polymorphic Variants and Multiple Sclerosis in Orkney"

3) "Serum Immunoglobulin Levels in Multiple Sclerosis in Orkney"

The investigators anticipate publication of these manuscripts in the *British Journal of Preventive and Social Medicine* in the fall of 1977. These manuscripts are as yet not available for review; however, recently the information became available that the findings do not support heredity as an etiologic factor.

Significance to the NINCDS Program and Biomedical Research: (See this item as stated for contract N01-NS-4-2321.)

Proposed Course of the Project: The contract expired on June 26, 1977. The following reports required by the contract are still outstanding:

(1) Church burial data to complete Shetland pedigrees.

(2) Receipt of the three manuscripts pertaining to genetic analysis of multiple sclerosis in Orkney as listed under "Major Findings".

(3) Shetland pedigree analysis.

(4) The final report on the genetic aspects of multiple sclerosis among the inhabitants of Shetland and Orkney Islands.

At this time no significant reason exists to doubt that the outstanding reports will be obtained in a reasonable period of time. In accordance with the terms of the contract, as soon as satisfactory reports are received, payment of the remaining $19,524.18 will be made.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1977

NATIONAL ANALYSTS, INC. (N01-NS-4-2335)

Title: Survey of the Incidence, Prevalence, and Costs of Multiple Sclerosis

Contractor's Project Director: Lorna B. Sherman

Current Annual Level: $73,191

Objectives: The primary objective of this survey is to produce national estimates and accompanying measures of precision for the incidence, prevalence, and economic costs of multiple sclerosis.

Major Findings: To date, the first stage of the study, the data collection of cases of multiple sclerosis identified by hospitals and physicians during the six year period from January 1970 to December 1975, has been completed.

Significance to the NINCDS Program and Biomedical Research: This survey is important for two reasons. First, it provides national estimates of the incidence, prevalence, and economic costs of multiple sclerosis. These estimates are especially useful to NINCDS for purposes of program planning and allocation of funds. Second, the survey will demonstrate to health investigators the value of probability sampling as a tool in sample selection. Probability sampling is the only general method available which can provide a measure of the precision of an estimate. Though this method is widely used in other areas, it is largely neglected in health studies. This survey, and the others of the NINCDS Survey Program, will demonstrate that probability sampling is both desirable and feasible for certain types of health studies.

Proposed Course of the Project: Estimates of incidence and prevalence will be calculated for different groups of patients. A sample of patients will be drawn for inclusion in the patient portion of this study. Patients will be interviewed to obtain information relevant to both direct and indirect costs of the disease. A second interview will inquire into health status during the 90-day period, so that an attempt can be made to relate fluctuations in costs to changes in activity of the disease.

Concurrent with the patient phase of the study, a sub-sample of patients (other than those selected for interview) nominated only by physicians in the non-certainty stratum will be drawn. The nominating physician will then be offered an opportunity for that patient to have a consultation with a neurologist, at no cost to the patient. This phase will be used to evaluate the accuracy of the diagnosis when it is reported by a non-neurological specialist.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1977

RESEARCH TRIANGLE INSTITUTE (N01-NS-4-2334)

Title: Survey of the Incidence, Prevalence and Costs of Head and Spinal Cord Injury

Contractor's Project Director: Dr. Daniel G. Horvitz

Current Annual Level: $287,011

Objectives: The primary objective of this survey is to produce national estimates and accompanying measures of precision for the incidence, prevalence, and health costs of head and spinal cord injuries for the calendar year 1974. In addition to overall national estimates, other estimates of head and spinal cord injuries will be produced for special domains of study. These domains are defined in terms of the following variables: age, race, sex, marital status, region of the country (four major regions), or external cause of injury. The two most important external causes of injury are automobile accidents and falls.

Major Findings: The objectives of this survey have been realized and the findings have been presented to NINCDS in the form of a final report. NINCDS staff will edit the final report and will submit it for publication as a supplement to a major journal.

Significance to the NINCDS Program and Biomedical Research: This survey is important for two reasons. First, it provides national estimates of the incidence, prevalence, and economic costs of head and spinal cord injury. These estimates are especially useful to NINCDS for purposes of program planning and allocation of funds. Second, the survey will demonstrate to health investigators the value of probability sampling as a tool in sample selection. Probability sampling is the only general method available which can provide a measure of the precision of an estimate. Though this method is widely used in other areas, it is largely neglected in health studies. This survey, and the others of the NINCDS Survey Program, will demonstrate that probability sampling is both desirable and feasible for certain types of health studies.

Proposed Course of the Project: The study is completed and the final report has been submitted. The contract will be extended for three months at no additional cost to the Government to permit staff of the Research Triangle Institute to assist NINCDS with the preparation of the journal supplement.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1977

WESTAT, INC. (N01-NS-4-2336)

Title: Survey of Intracranial Neoplasms

Contractor's Project Director: Dr. Carolyn A. Eldred

Current Annual Level: $62,431

Objectives: The primary objective of this survey is to produce national estimates and accompanying measures of precision for the incidence, prevalence, and economic costs of intracranial neoplasms.

Major Findings: The objectives of this survey have been realized and the findings have been presented to NINCDS in the form of a final report. NINCDS staff will edit the final report and will submit it for publication as a supplement to a major journal.

Significance to the NINCDS Program and Biomedical Research: This survey is important for two reasons. First, it provides national estimates of the incidence, prevalence, and economic costs of intracranial neoplasms. These estimates are especially useful to NINCDS for purposes of program planning and allocation of funds. Second, the survey will demonstrate to health investigators the value of probability sampling as a tool in sample selection. Probability sampling is the only general method available which can provide a measure of the precision of an estimate. Though this method is widely used in other areas, it is largely neglected in health studies. This survey, and the others of the NINCDS Survey Program, will demonstrate that probability sampling is both desirable and feasible for certain types of health studies.

Proposed Course of the Project: The study is completed and the final report was submitted by June 30, 1977.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1977

WESTAT INC. (N01-NS-6-2333).

Title: Nationwide Study of Stroke

Contractor's Project Director: Morton Robins

Current Annual Level: $230,283

Objectives: The primary objective of this survey is to produce national estimates and accompanying measures of precision for the incidence prevalence, and economic costs of stroke. It would be accomplished by devising and testing a method for carrying out the survey, and for presenting the statistical results of the survey, including the sampling errors.

Major Findings: Phase I. The first part of the survey has been completed in a group of 26 hospitals in the mid-west. OMB clearance has been obtained for the remainder of the study hospitals. A clinical algorithm has been developed to apply to the abstracted hospital records so as to classify the records properly according to type of stroke and degree of confirmation of the diagnosis by the medical records.

Significance to the NINCDS Program and Biomedical Research: This survey is important for two reasons. First, it provides national estimates of the incidence, prevalence, and economic costs of stroke. These estimates are especially useful to NINCDS for purposes of program planning and allocation of funds. Second, the survey will demonstrate to health investigators the value of probability sampling as a tool in sample selection. Probability sampling is the only general method available which can provide a measure of the precision of an estimate. Though this method is widely used in other areas, it is largely neglected in health studies. This survey, and the others of the NINCDS Survey Program, will demonstrate that probability sampling is both desirable and feasible for certain types of health studies.

Proposed Course of the Project: For each stroke discharge selected in the sample hospitals the medical records will be abstracted and pertinent information on the patient's demographic characteristics, the diagnostic categorization of the stroke, date of onset, medical history of previous strokes or TIA's, clinical signs and symptoms, diagnostic findings, treatment and outcome of hospital care will be recorded. In addition, the hospital's business office will be asked to furnish data on the direct medical charges to the patient or third party payer on only those cases discharged during 1975. Moreover, for those discharged patients in the sample that were presumed to have had an initial stroke attack, their survival status will be determined as of December 31, 1975.

A subgroup of surviving patients (or close relatives) will be interviewed to obtain information on direct expenditures related to the stroke incurred during 1975 by the patient or the family. Additional facts will also be sought on other aspects of economic impact of a stroke on the patient and the family.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1977

UNITED STATES BUREAU OF THE CENSUS (YO1-NS-70031)
UNIVERSITY OF MISSISSIPPI (NO1-NS-7-2357)

Title: Survey of Major Neurological Disorders in Copiah County Mississippi

Contractor's Project Director: Mr. Evan H. Davey (Bureau of the Census); Dr. Armin F. Haerer (University of Mississippi)

Current Annual Level: $137,000 (Bureau of the Census); $19,601 (University of Mississippi)

Objectives: The overall objective of the proposal is to establish the prevalence of six major neurological and developmental disorders (cerebrovascular disease, convulsive disorders, cerebral palsy, mental retardation, Parkinson's disease, and dementia) in a well-defined population of southern blacks and whites. The initial phase of the study will be the development of practical and accurate methods and instruments to detect these disorders in a survey of the population. A Pilot Study will be conducted to test these methods and instruments in a community outside of Copiah County but comparable to communities found there.

Major Findings: To date, the methods and instruments have been developed. The Pilot Study will be conducted in August, 1977 after the OMB Clearance and Human Subjects Clearance have been obtained.

Significance to the NINCDS Program and Biomedical Research: At present, there are no adequate data on the prevalence of the six disorders of interest among southern blacks and whites in the United States. A number of studies suggest that stroke is more common among the black population. Mortality data and a few morbidity studies suggest that Parkinson's disease is less common among blacks. A biological explanation of this observation is that both melanin and dopamine are involved in the same metabolic pathway. Dopamine-deficiency in the basal ganglia has been found in patients with Parkinson's disease and is the rationale for treatment of this condition with L-dopa. Blacks have a higher concentration of dopamine in the basal ganglia than whites which could explain a lower frequency of Parkinson's disease. On the other hand, it may be that blacks with this condition do not seek medical care or receive inadequate care. Mortality tabulations, with all of their biases, suggest that blacks have a predominance of epilepsy and cerebral palsy, but this requires confirmation with morbidity data. The magnitude of the dementia problem has not been studied in any U.S. population and Copiah County will provide some indication as to whether there is a racial and sex differential in the frequency of this group of conditions.

Proposed course of the project: The field operations for the Main Study, as well as for the Pilot Study, are divided in two types of operations. The first is a household screening operation which will be conducted by the Bureau of the Census. Residents of the study area will be screened in their homes by means of a questionnaire administered by lay interviewers who are trained and supervised by the Bureau of the Census. The second type of operation is the examination of persons suspected of having one or more of the disorders of interest on the basis of responses given to questions from the screening questionnaire. The University of Mississippi will provide senior, board-certified neurologists to accomplish the neurological examinations and to record the medical findings on forms designed especially for this study. After the close of the field operations, the data will be sent to the Bureau of the Census for processing. When the data tapes and files are available for analysis, the Project Director and Associate Project Director from the University of Mississippi will assist NINCDS in the data analysis and the preparation of scientific papers based on the findings of the survey.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1977

CONTRACTOR TO BE SELECTED (RFP-NIH-NINCDS-77-05
NATIONAL CENTER FOR HEALTH STATISTICS (Y01-NS-7-0030)

Title: Comprehensive Disease Statistics Survey

Contractor's Project Director: To be selected;
NCHS - Dr. Monroe Sirken

Current Annual Level: Contractor - $200,000
NCHS - 25,000

Objectives: The objectives are to test the feasibility of obtain- hospital incidence and prevalence data for cases identified from abstracted hospital records of a number of neurological and other disorders, from a redesigned Hospital Discharge Survey of the NCHS. A key objective of a successful pilot study would be to develop a survey program that would permit the annual collection of data on these disorders in order to develop trends of their incidence and prevalence.

The national sample of short-stay hospitals would provide a stable base for special studies. These studies would include methodological problems such as multiplicity. It would also provide an unbiased sample of patients, for periodic studies of special interest such as costs of illness, degree of disability, duration of illness, etc. Comparability of data collection methods, and protocols from the same sample of short-stay hospitals, would also permit comparison across disease lines.

Major Findings: The study has been designed and the algorithm for several disorders have been prepared. In September, 1977 the selected contractor will prepare the detailed protocols for the feasibility study. NCHS has been involved in a cooperative effort with NINCDS to plan and design the study and to work on the many methodological and statistical problems involved in the survey.

Significance to the NINCDS Program and Biomedical Research: NINCDS has current contracts for surveys of four neurological disorders, and other surveys are underway and in the planning stage. For several reasons there is a need to consider a more comprehensive approach to the collection of disease statistics. First, there is a considerable degree of redundancy in the present approach, both within NINCDS, and, probably, across Institute boundaries. Redundancy leads to higher than necessary costs associated with the collection of disease statistics data. Second, the present approach leads to delays in obtaining current information, since there are a limited number of surveys which can be conducted at any one time. Third, the methods and protocols used by each contractor differ, and this affects the comparability of data across disease lines. Fourth, and perhaps most important, these data provide planning information based on a limited time period, when in fact trend data, obtained on an annual, prospective basis, would better serve the program planning and program evaluation functions.

The development of a comprehensive system for the collection of disease statistics on a wide variety of diseases would be of great value to NINCDS and other NIH Institutes for it would eliminate the four above mentioned major problems.

This proposal would establish a cooperative and joint relationship between NCHS and NINCDS, and would provide for an NCHS collection of national health statistics of considerable interest to NINCDS, and potentially, to other NIH Institutes. It would, to the extent that incidence and prevalence data can be obtained from records at short-stay hospitals, supplant NINCDS data collection efforts. NINCDS would continue to analyze the data collected to meet its own program planning needs.

Proposed Course of the Project: This project is a joint venture. The contractor will be responsible for only part of the study. The contractor will develop the methodology and protocols for the feasibility study and the pilot study, will provide the field staff, and will conduct the study and process the data. The disease algorithms will be prepared by NINCDS staff with the aid of other participating NIH Institutes. The sampling plan, counting rules for non-duplication of cases, estimation of sampling variances, and coordination with participating hospitals will be conducted by the National Center for Health Statistics, HRA, under a separate interagency agreement.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OBE, NINCDS
Fiscal Year 1977

UNIVERSITY OF TEXAS AT HOUSTON, SCHOOL OF PUBLIC HEALTH (N01-NS-3-2304)

Title: Stroke Risk Factors in Panama

Contractor's Project Directors: Dr. Darwin Labarthe and
Dr. Reuel Stallones

Project Officer: Dr. Bruce S. Schoenberg

Amount of Contract: This fiscal year represented an extension without additional funds.

Objective: During the past year a study of stroke risk factors among various black populations in Panama was completed. Stroke and hypertension are known to be higher in blacks than whites. The purpose of this particular investigation was to examine "genetically distinct" black populations, each living in an urban and rural setting, to determine environmental and genetic factors in these conditions. Information on family history, life style, and cardiovascular disease was collected. In addition, blood pressure measurements, anthropometric measurements, electrocardiographic examinations, physical examinations, and laboratory examinations (including serum lipids and 20 genetic markers) were carried out. Despite numerous political difficulties, the group was able to collect what appeared to be good information on a group of rural English-speaking blacks and a group of rural Spanish-speaking blacks. These data were analyzed in this fiscal year. Although there is good descriptive population-based data on rural English speaking blacks, the fact that the survey was incomplete in other areas makes comparisons among groups hazardous.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OBE, NINCDS
Fiscal Year 1977

NATIONAL ACADEMY OF SCIENCE (PH-43-64-44)

Title: Epidemiologic Study of Amyotrophic Lateral Sclerosis and Other Motor Neuron Diseases

Contractor's Project Directors: Dr. Gilbert Beebe and
Dr. John Kurtzke

Project Officer: Dr. Bruce S. Schoenberg

Amount of Contract: This fiscal year represented an extension without additional funds.

Objective: The purpose of the study is to identify risk factors for the development of motor neuron disease and to determine the natural history of this group of diseases. The project is divided into three parts. In the first section of the investigation, World War II service medical records from 500 veterans who died of amyotrophic lateral sclerosis in the years 1963 through 1967 were examined to measure potential risk factors for the subsequent development of motor neuron disease. Appropriate controls were selected from service life insurance holders. The second part of the study involved an extensive interview survey of veterans currently hospitalized for motor neuron disease to again assess clues to etiology. Brain tumor patients were selected as matched controls. A final aspect of the study involves a follow-up of veterans discharged from VA hospitals with a diagnosis of amyotrophic lateral sclerosis to determine the natural history of the disease. With regards to the first part of the study, analysis has been completed and results were as follows: Comparisons were made with attention to three- and four- digit detail of codes for preservice occupation and industry, defects found at induction, military occupation, and diagnoses in service. There was a slightly suggestive (P=.10) deficit of blacks with ALS, and several differences in preservice occupations, but a very clear-cut excess of trauma in the ALS cases, with 163 diagnoses vs. 115 in the controls (P .01). The preservice histories of trauma and of surgical operations also differentiated the two groups. There was a suggestive excess of men in the ALS group giving a preservice history of active participation in baseball, but no significant difference in aggregate scores of preservice sports activity.

Cooperation for part 2 - the interview of ALS patients and controls was poor and far less data were obtainable than originally anticipated. With reference to phase 3 of the study, preliminary analysis revealed that the diagnosis of ALS was frequently in error. Because of the significant problems entailed with parts 2 and 3, the Biometry and Epidemiology Program Review Committee of NINCDS requested that these efforts be terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02237-01 OBE

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development and Design of a Pilot Study for a National Survey of Epilepsy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William Weiss, Chief, OBE, NINCDS

PI: Bernard H. Kroll, Associate Chief, OBE, NINCDS

Other: Frederic Weinfeld, Head, Section on Disease Statistics Surveys, OBE, NINCDS

COOPERATING UNITS (if any)

W. Allen Hauser, Dept. of Neurology, St. Paul-Ramsey Hosp., St. Paul, Minn. 55101
Joseph Steinberg, Survey Design, Inc.

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.50	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This pilot was initiated to develop a new and untried method of ascertaining the incidence and prevalence of epilepsy. The previously used methods have serious deficiencies and this proposal seeks to remedy them. The goal is to use pharmacies filling anti-convulsive prescriptions to lead to the physicians providing care and thus to the epileptics. By using nationwide probability sampling techniques valid estimates of the U.S. epileptic population should be made.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02238-01 OBE

PERIOD COVERED April 1, 1977 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Computerized Interactive Data Bank for Comprehensive Stroke Centers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Selma C. Kunitz, Acting Head, Systems and Programming Section, OBE, NINCDS

Other: Kenneth Elsner, Systems Analyst, OBE, NINCDS
Sylvia Edelstein, Programmer, OBE, NINCDS

COOPERATING UNITS (if any)

Dr. Murray Goldstein, Ms. Jean Benedict,
Stroke and Trauma Program, NINCDS

LAB/BRANCH
OBE

SECTION
Systems and Programming

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of developing a computerized interactive databank for the Comprehensive Stroke Centers is to provide a model for neurological diseases which will aid both the clinician and researcher in the treatment, prognosis, rehabilitation, and possible prevention of such diseases. The objectives of the project are: A. To develop a uniform method of data collection utilizing standard clinical nomenclature and standardized input format for patient histories, diagnosis, and treatment. B. To implement an interactive comprehensive data base system for facilitating patient care, clinical research, and health education. C. To establish a national stroke database network, enabling pooling of clinical data among institutions, collaborative inter-institutional studies, rapid access to large quantitites of clinical data, and computer consultation. D. To demonstrate the feasibility of such a network, including the computer aspects, the collaboration among a number of institutions, to serve as a model for neurological diseases and disorders.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02239-01 OBE

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Design of Stroke and Convulsive Disorder Questionnaires

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bernard H. Kroll, Associate Chief, OBE, NINCDS

Other: Bruce Schoenberg, Head, Section on Epidemiology, OBE, NINCDS

William Weiss, Chief, OBE

COOPERATING UNITS (if any)

Clint Burnham, Health Interview Survey, NCHS
W. Allen Hauser, Dept. of Neurology, St. Paul-Ramsey Hospital
St. Paul, Minn. 55101

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS: .5	PROFESSIONAL: .5	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The design and field testing of questionnaires to be used as a supplement to the NCHS Health Interview Survey (HIS). The first was designed to determine the persons with some degree of stroke, diagnosed or undiagnosed; and hospitalization. This questionnaire was included in the 1977 HIS. A second has been designed to measure those persons with convulsive disorder and is being proposed for the 1978-79 HIS.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02240-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Descriptive Epidemiology of Dementia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., OBE, NINCDS

COOPERATING UNITS (if any)

Wayne Massey, M.D., Neurologist, National Naval Medical Center

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS. Bethesda. Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the prevalence of dementia in well-defined populations are planned. Protocols have been developed and are currently being tested.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02241-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of Pre-existing Cardiac Disease and/or Hypertension as Risk Factors for Subsequent Completed Stroke and Transient Ischemic Attacks

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg, Devera G. Schoenberg, and Dr. Jack P. Whisnant (Department of Neurology, Mayo Clinic)

COOPERATING UNITS (if any)

LAB/BRANCH Office of Biometry and Epidemiology

SECTION Epidemiology

INSTITUTE AND LOCATION NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation is aimed at evaluating the effect of heart disease and hypertension as potentially treatable precursors of stroke and transient ischemic attacks. The objectives of the study are to determine the following: (1) the risk of stroke and transient ischemic attacks in individuals with heart disease and/or hypertension as compared to the risk in individuals without these conditions; (2) whether the existence of pre-existing heart disease and/or hypertension affects the type of stroke and whether it affects survival following stroke; and (3) whether there is a particular time interval following the onset of heart disease or hypertension during which an individual is at high risk for stroke. Data collection for this study has been ongoing since 1973, and it is estimated that an additional year will be required to complete the investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02242-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less) The Association Between Myasthenia Gravis and Multiple Sclerosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg and Dr. Drake Duane
(Department of Neurology, Mayo Clinic)

COOPERATING UNITS (if any)

LAB/BRANCH Office of Biometry and Epidemiology

SECTION Epidemiology

INSTITUTE AND LOCATION NINCDS, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There have been numerous reports of the association between myasthenia gravis and multiple sclerosis. The Mayo Clinic experience with these two diseases will be reviewed for cases of association, and this experience will be analyzed in an attempt to determine whether this association occurs more often than might be expected simply on the basis of chance occurrence.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02243-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cerebrovascular Disease in Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg, Devera G. Schoenberg and Dr. James F. Mellinger (Department of Neurology, Mayo Clinic)

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.15	PROFESSIONAL: 0.15	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Mayo Clinic experience with cerebrovascular disease in children was examined for the years 1965 through 1974. The medical record linkage system available for all medical facilities caring for the Rochester population makes it possible, for the first time, to establish an incidence rate for cerebrovascular disease in children. These data have been collected and are currently being analyzed. Four papers on this subject have been prepared and submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02244-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Unusual Patterns of Cerebrovascular Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg and Dr. Jack P. Whisnant
(Department of Neurology, Mayo Clinic)

COOPERATING UNITS (if any)

LAB/BRANCH Office of Biometry and Epidemiology

SECTION Epidemiology

INSTITUTE AND LOCATION NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Unusual clinical patterns of cerebrovascular disease in the experience of the Mayo Clinic are being examined (e.g. the occurrence of more than 15-20 transient ischemic attacks/day). Such material is being critically evaluated in an attempt to link unusual clinical presentations with specific pathological lesions.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02245-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cerebrovascular Disease During Pregnancy and the Postpartum Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg and Dr. Burton Sandok
(Department of Neurology, Mayo Clinic)

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study will attempt to define the incidence and nature of cerebrovascular disease in the young female as well as the incidence of such events in the pregnant and non-pregnant population. This may provide us with information to better understand the increased risk of cerebrovascular events presumed to occur in association with the use of oral contraceptives. This investigation is just getting under way.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02246-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Alzheimer's Disease Questionnaire and Case-Control Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., Head, Section on Epidemiology, OBE, OD, NINCDS

Other: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, OD, NINCDS

COOPERATING UNITS (if any)
Albert Heyman, M.D., Professor, Deptartment of Neurology

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
Duke University, Durham, North Carolina

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Development of a suitable epidemiologic protocol and questionnaire for a case-control study of Alzheimer's disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02295-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
ALS Registry & Questionnaire

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., Head, Section on Epidemiology, OBE, OD, NINCDS

Other: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, OD, NINCDS

COOPERATING UNITS (if any)
Bonnie Klein, M.P.H., Epidemiologist
ALS Society of America, Los Angeles, California

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Md.

TOTAL MANYEARS: 0.05	PROFESSIONAL: 0.05	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Development of operational systems and a series of questionnaires to be used in an ALS registry being implemented by the ALS Society of America.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02296-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Registry for Creutzfeldt-Jakob Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., OBE, NINCDS

COOPERATING UNITS (if any)

Charles Poser,

Department of Neurology, University of Vermont

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Development of a protocol for the implementation of a registry for Creutzfeldt-Jakob disease to include epidemiologic, pathologic, and clinical information.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02297-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Analyses of U.S. Mortality Data for Neurological Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Judith E. Hogg, M.D., Neurologist, OBE, NINCDS

Other: Bruce S. Schoenberg, M.D., M.P.H., Head
Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)
E. Wayne Massey,
U.S. National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.03	PROFESSIONAL: 0.03	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Analysis of recent U.S. mortality data with regards to neurological data in order to examine the geographic distribution and identify areas with particularly high or particularly low death rates. These preliminary analyses may lead to etiologic clues.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02298-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mortality in 33 Countries from Diseases of the Nervous System.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., Head
Section on Epidemiology, OBE, OD, NINCDS

COOPERATING UNITS (if any)
E. Wayne Massey, M.D., Neurologist, U.S. National Naval Hospital Center, Bethesda, Maryland

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Md.

TOTAL MANYEARS: 0.05	PROFESSIONAL: 0.05	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Analysis of international mortality data on neurological disease collected through the auspices of the world health organization. Portions of this work will be presented at the 11th World Congress of Neurology.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02299-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Analysis of National & International Mortality Data for Huntington's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Judith E. Hogg, M.D., OBE, NINCDS
Other: Bruce S. Schoenberg, M.D., M.P.H., OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

At the request of the Huntington's Disease Commission, the Section on Epidemiology is preparing a set of standard mortality indices for Huntington's disease in the United States and for other countries where suitable data are available. The results of these studies will be published as part of the Commission's final report.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02300-01 OBE

PERIOD COVERED July 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Life Expectancies in Neurological Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bernard H. Kroll, Associate Chief, OBE, NINCDS
Other: Bruce Schoenberg, Head, Section on Epidemiology, OBE, NINCDS
Robert Richter, Mathematician, Section on Systems Design and Data Processing, OBE, NINCDS

COOPERATING UNITS (if any)

Mayo Clinic, Rochester, Minnesota

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, Bethesda, Md. 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study uses a review and abstraction of data from records for a selected group of neurological disorders. It obtains the items of data which will develop onset of the disorder, duration, date and cause of death or current status. These data will be used to construct modified life tables to estimate the expectation of life after diagnosis, the survival curve and morbidity and severity estimates.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02301-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Space/Time Clusters of Neurological Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., Head,
Section on Epidemiology, OBE, OD, NINCDS

Other: Judith E. Hogg, M.D. Neurologist, Section
on Epidemiology, OBE, OD, NINCDS

Tatiana Kudrjavcev, M.D., Neurologist,
Section on Epidemiology, OBE, OD, NINCDS

COOPERATING UNITS (if any)
Richard Kaslow, M.D., Center for Disease Control
Atlanta, Georgia

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Md.

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Development of protocols and strategies for the investigation of space/time clusters of neurological disease. This also involves studies of neurological diseases resulting from environmental toxins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02302-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

International Programs in Neuroepidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., OBE, NINCDS

COOPERATING UNITS (if any)
Neurosciences Program, World Health Organization,
Geneva, Switzerland

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Development of protocols and strategies to study the epidemiology of neurological disease in developing countries. Emphasis will be placed on uniformity and standardization so that results of investigations can be compared.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02303-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Racial Differentials in the Occurrence of Brain Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., OBE, NINCDS

COOPERATING UNITS (if any)
Lawrence W. Mahalak, Jr., Division of Neurology,
University of Mississippi

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

African studies suggest that the brain tumor experience of blacks is both quantitatively and qualitatively different from that of whites. No data are yet available for brain tumors on a well-defined population of blacks in the United States. However, the experience of a southern medical center caring for a large black population will be examined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02304-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Extracranial Malignancies Metastatic to Intracranial Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg, Dr. R. Jean Campbell (Department of Pathological Anatomy, Mayo Clinic) Dr. Albert Heck (Department of Neurology, University of Maryland), and Dr. R. Simon, Berkeley, California

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study represents analysis of a series of cases in which an extracranial malignancy metastasized to a primary intracranial neoplasm. The pathological specimens are being carefully examined in hopes of providing clues to the etiology of this phenomenon. A paper is being prepared for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02305-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Descriptive Epidemiology of Primary Intracranial Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg, Dr. Barbara W. Christine (Connecticut State Department of Health) and Dr. Jack P. Whisnant (Department of Neurology, Mayo Clinic)

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The descriptive epidemiology of primary intracranial neoplasms (including tumors of the pituitary gland) has been studied with regard to incidence, survival, and the association of these neoplasms with primary malignancies of other sites. A paper describing incidence patterns by age, over time, and by histologic type is being prepared for publication. Data on primary intracranial neoplasms from the Third National Cancer Survey have been obtained from the National Cancer Institute and will be similarly analyzed. These data, however, include only those neoplasms specified as "malignant".

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02306-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Resolution of Reported Differences in the Incidence of Primary Intracranial Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Bruce S. Schoenberg, Dr. Barbara W. Christine, (Connecticut State Department of Health) and Dr. Jack P. Whisnant (Department of Neurology, Mayo Clinic)

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Age-specific incidence curves from the state of Connecticut reveal a small peak in childhood followed by a much taller, sharper peak with a maximum between ages 55 and 65. This also holds true for data collected from most large population-based registries. In contrast to this are the age-specific incidence curves for Rochester, Minnesota, which show a steady increase in incidence with increase in age. In addition, the reported incidence rate at any particular age for Rochester, Minnesota, is higher than comparable rates from other registries. The reasons for these discrepancies are currently being analyzed. A manuscript concerning the findings is being prepared for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02307-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Conference on Neurological Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: B.S. Schoenberg, Head, Epidemiology Section, OBE NINCDS

Other: F. Weinfeld, Head, Disease Statistics Surveys Section OBE NINCDS
E. Beadle, Statistician, Epidemiology Section, OBE NINCDS
J. Ellenberg, Head, Section on Math. Stat., OBE NINCDS
T. Kudrjavcev, Neurologist, Epidemiology Sec. OBE NINCDS
J. E. Hogg, Neurologist, Epidemiology Section OBE NINCDS
B. Nichols, Programmer, SDDPS OBE NINCDS
W. Weiss, Chief, OBE NINCDS

COOPERATING UNITS (if any)

D. Schoenberg, Research Epidemiologist, Bethesda, Md.

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, Md.

TOTAL MANYEARS: 0.77	PROFESSIONAL: 0.77	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An international two-day conference on neurological epidemiology was held in Washington, D.C., on May 15-17, 1977. Approximately 50 representatives from Asia, Africa, Europe, Latin America, and the U.S. attended. The various speakers and discussants discussed current knowledge in neuroepidemiology and stressed the applicability of this information to the practice of neurology and neurosurgery. A national one-day course on the principles of neurological epidemiology was offered. Members of OBE presented six papers at the Conference. The papers will be published in a book Neurological Epidemiology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02308-01 OBE

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

An Algorithm for a Morbidity Index

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T.C. Chen, Mathematical Statistician, OBE NINCDS

Other: B. H. Kroll, Associate Chief, OBE, NINCDS
William Weiss, Chief, OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS: .75	PROFESSIONAL: .75	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To develop a mathematically acceptable algorithm for the design of a morbidity index to permit comparisons of various diseases as to their impact on the persons affected, their families and society. The problem is being attacked in three ways, a review and critical analysis of the existing literature to select useful measures, a theoretical model of the problem with its mathematical interpretations and a proposed practical example of the model.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02309-01 OBE

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Statistical Methods in Neuronal Spike Train Analysis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.L. Yang, Research Fellow	OBE, NINCDS
	T.C. Chen, Mathematical Statistician	OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Microelectrode recording techniques have been widely used in neurophysiological, neuropharmacological and clinical neurological studies for probing cellular, electrical discharging behavior of nerve cells. The data generated from these recordings are often called spike-train data. There has been a number of statistical methods developed based on various models of the neuronal mechanism of impulse firing for spike-train data analysis. The validity of these methods needs to be examined for various purposes of application. In this study the currently available methods of spike-train data analyses were extensively reviewed and evaluated from a stochastic process point of view. Several optimal statistical tests for validating the use of these methods have been developed. A report of this study has been prepared for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02310-01 OBE

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Sensory-Decision-Theory Approach to the Measurement of Pain Components.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T.C. Chen, Mathematical Statistician OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to investigate the validity of the Sensory-Decision-Theory Approach to the measurement of two components - sensory discriminability and reporting bias - in the response of experimental pain. The mathematical relationship of the indices of sensory discriminability and reporting bias has been studied. The results of the investigation indicate that when sensory discriminability changes, the estimation of reporting bias by SDT approach is no longer appropriate. A Monte-Carlo study has been designed and will be carried out to examine the sampling behavior of these two indices under various statistical assumptions. A report of this study will soon be prepared.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02311-01 OBE

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Recovery of Corrupted EMI Data

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.J. Talbert, Statistician	OBE	NINCDS
Other:	R.A. Brooks, Physicist	SN	NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The nature of a process corrupting EMI scanner data was discovered by analysis of representative listings. The process involved spurious cyclic application of logical OR and NOT operations to the underlying bits of the binary data. The errors occurred in a contiguous 6-bit sequence in every consecutive 40-bit string in the files. Relying on an understanding of the corruption process and limiting values of the data, a method was devised by which the data could be restored sufficiently for clinical use. A lengthy and critical reconstruction was made of existing corrupted data.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02312-01 OBE

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Methodology for systematic statistical analysis of multiple antibody readings on matched controlled studies.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.J. Talbert, Statistician	OBE	NINCDS
	J.H. Ellenberg, Head, Section on Math. Stat.	OBE	NINCDS
Other:	J.L. Sever, Chief Infectious Diseases Branch	OBE	NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: .1	PROFESSIONAL: .1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A system was developed for analysis of multiple antibody response data in paired sera from matched control studies. The system includes an exacting data quality control step and comprehensive statistical testing to compare abnormals with controls, and to detect and flag seroconversions. For chi-square tests an interval function was developed which automatically lumps cells to prevent low cell frequencies, in a logical manner. It was necessary to extend existing Kolmogorov-Smirnov tables of critical values to accommodate the frequencies which could be encountered in studies of this type.

ANNUAL REPORT
Director's Report
July 1, 1976 through September 30, 1977
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

The Extramural Activities Program (EAP), NINCDS, was organized in July 1975, to serve as the Institute coordination and focal point for science administration, fiscal management and administrative management of its grant, fellowship and research contract activities. The structure of EAP includes units responsible for extramural committee management, data reporting and analysis, scientific merit review, contract management, grants management and program support services including processing; executive focal points also have been established for the research grant programs, the research contract programs and the manpower development programs. Fiscal Year 1977 was characterized by the development and stabilization of these organizational units, the definition of responsibilities, the development of operational patterns and the conduct of Institute extramural activities; this latter point must be emphasized for as these important organizational changes were being developed and implemented, the extramural program activities of the Institute were in full operation and had to be maintained at high levels of effectiveness and efficiency. I am pleased to report that both the required organizational changes were able to be implemented and ongoing program activities continued with a minimum of confusion or disruption. Issues that require further attention include functional elaboration of the responsibilities of program area staff and EAP staff, relationships and relative responsibilities of the Institute Program Planning Office and the EAP Data and Report Unit, and the relative roles of EAP staff and program area staffs to the activity of the National Advisory Council.

A major physical move of importance and consequence was accomplished in March, 1976. This move placed all EAP personnel and all program area personnel in the same building. As with any physical relocation, the move is a mixture of blessings and horrors. With time and the development of new work patterns, most problems will be resolved. A small number of renovations are critically required; improvement of poor lighting resources now being justified by antediluvian lighting standards need attention, and improved messenger services between the Federal Building, the Westwood Building, and the NIH campus require development.

An over-riding problem that seriously affects all extramural programs is that, although the total NINCDS budget has increased about $20 million over the past five years, in purchasing power the budget has actually decreased about $23 million. Added to this there was a real problem in keeping accurate budgetary records on commitments and funds available in each of the Program Areas. Part of the reason for this difficulty seemed to be an inability to obtain accurate predictions of future needs for new and renewal contracts. Since grant and contract funds are all in the same allocation for each Program Area, inaccurate information in regard to future needs for contracts resulted in inaccurate predictions in regard to the availability of funds for grants. Also, for reasons not entirely clear there apparently was a much larger increase than anticipated in committed funds for grants as the year progressed. During this period, relying upon innovative management techniques and excellent rapport with the scientific community, staff focused on maintaining the core research program and the development of alternatives for program implementation at an appropriate time. Conceptual approaches to program implementation at a flat level budget are being developed and pilot activities initiated to pre-test these concepts.

A major change in personnel occurred during the past year when Dr. Murray Goldstein resigned his appointment as Director, Extramural Activities Program to give full time to being Director of the Stroke and Trauma Program. Dr. O. Malcolm Ray, Deputy Director, was Acting Director, Extramural Activities Program, for most of the year until Dr. K. Kenneth Hisaoka was officially appointed as the new Director. Dr. Hisaoka was formerly Deputy Associate Director, Extramural Programs, NIDR.

In summary, several tangible problems of space and resources require solution; a number of less tangible problems involving the working relationships among staff require more precise definition and focused attention. The tardiness of appropriation decisions and the relative insecurity of appropriation and personnel resources have delayed the initiation of needed program implementation and the maintenance of ongoing programs. However, by relying upon both the staff's excellent rapport with the neuroscience and communicative science communities and the use of innovative administrative techniques, the core program was continued with a minimum of disruption or uncertainty.

ANNUAL REPORT
July 1, 1976 through September 30, 1977

EXTRAMURAL ACTIVITIES (RESEARCH GRANTS)
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

This portion of the NINCDS Annual Report is intended as an overall summary of administrative, logistical and personnel problems and developments as they pertain to the Extramural Activities Program, particularly research grants. For purposes of cohesiveness, however, other activities (training awards, contracts, etc.) are mentioned although they are discussed in more detail elsewhere.

The research grant, contract and training programs of the NINCDS are focused on the identification, stimulation, and support of essential research problems aimed at the improved diagnosis, treatment, and prevention of disorders of the nervous system, the neuromuscular apparatus, the ear, and human communication. They include disorders of the young (cerebral palsy, epilepsy, learning disabilities) of adulthood (head and spinal cord injury, multiple sclerosis, brain tumors) and of the aged (stroke, parkinsonism, otosclerosis). The administrative instruments used to accomplish these purposes include research projects, research program projects, clinical research centers, outpatient clinical research projects, specialized research centers, research career awards, research career development awards, teacher-investigator awards, institutional research fellowship awards, individual research fellowship awards and contracts.

The following Table shows the number of research grant applications considered by the Council at its spring meetings in recent years.

JUNE '71	JUNE '72	JUNE '73	JUNE '74	JUNE '75	SEPT. '76*	MAY '77
352	398	467	428	481	493	643

*Comparable to a spring meeting on the basis of the new fiscal year.

From June 1971 to September 1976, the number of applications reviewed has increased 40 percent with rather regular gradual increases from year to year. This is attributed primarily to the effectiveness of the research training programs of the Institute in which the output of fully trained investigators has only in recent years reached its full potential. The very large increase in May 1977 is probably due largely to the fact that for the past year or two it has been possible to fund only 25-30 percent of the approved applications. A large proportion of the unfunded applicants reapply, thus increasing the number of applications to be reviewed.

The following Table shows the number of research grants awarded and the total amounts of funds expended (in millions) each year for the past 10 years.

	FY '68	FY '69	FY '70	FY '71	FY '72
NUMBER	1,780	1,798	1,267	1,256	1,280
DOLLARS	$65.1	$67.8	$48.8	$48.8	$64.2

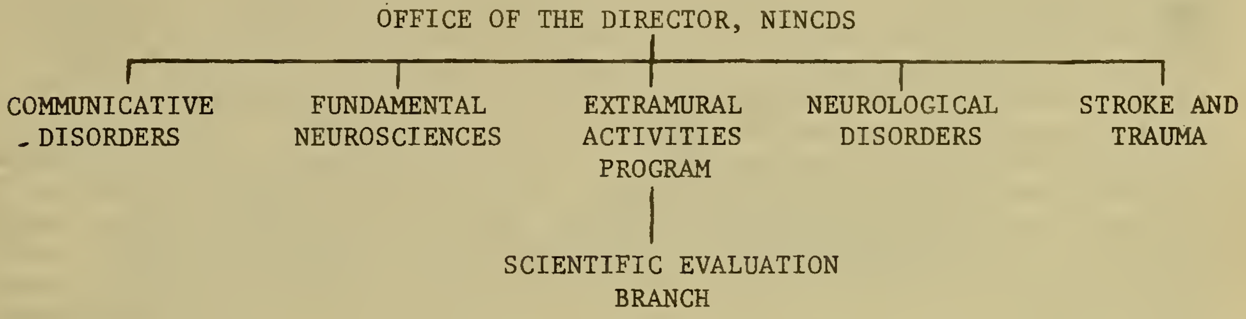
	FY '73*	FY '74	FY '75	FY '76	FY '77**
NUMBER	1,056	1,065	1,221	1,200	1,075
DOLLARS	$62.4	$70.0	$86.6	$90.4	$94.9

*Exclusive of Impounded Funds **Exclusive of Transition Quarter

The largest change was in FY '70 when all research on vision was removed from this Institute to form the National Eye Institute. At that time the number of grants decreased by 29% and the amount of funds by 28%. Until FY '72 the number of projects supported has been maintained at approximately the same level each year despite a marked increase in the cost of all aspects of doing research. This was made possible in part by modest increases in the amounts of funds available, but primarily by the fact that beginning in FY '70 every research grant, competing and committed, was subject to a negotiated reduction. All grants were negotiated downward by an amount which hopefully would still allow the work to proceed, although productivity doubtless was reduced because costs increased each year. These reductions, in effect, made additional funds available for competing proposals in the amounts of $7.0 million in FY '70, $5.0 million in FY '71 and $3.0 in FY '72. FY '72 was the last year in which such reductions were made because the National Institutes of Health decided that it was preferable to support fewer and better projects adequately than to support more projects with insufficient funds to do the work properly. The number of projects supported immediately decreased by 16%. It should be emphasized, however, that even after reducing each grant, funds were sufficient to support only the better half of approved proposals. Some increase in funds in FY '75 and FY '76 made it possible to bring the numbers of grants back almost to the number awarded in FY '70 after all of the projects on vision were transferred to the National Eye Institute. Perhaps it should be mentioned that for many years the approval rate for grant applications by the Initial Review Groups was almost exactly 50%. During the past 3-4 years this has increased to about 65%. We believe this is due to the fact that an increasing number of disapproved applications and many approved, but unfunded proposals, with the help of comments from the Initial Review Groups are being resubmitted in an improved form. Funds available this year permit supporting only about 25% of approved proposals.

For many years the Extramural Program was organized on the basis of a Research Grants Branch and a Training Grants and Awards Branch. During FY '74 the activities were reorganized along more functional programmatic lines consisting of a Regular Programs Branch and a Special Programs Branch. The primary responsibility of the former was the planning, development and operation of the investigator-initiated research grant programs. The primary responsibility of the Special Programs Branch was the planning, development and operation of the targeted grant programs including program projects, clinical research centers, special projects, training programs and manpower studies.

During FY '76 the entire extramural effort of the Institute was reorganized and the complete grants and training activities were moved (March 1976) from the Westwood Building to the Federal Building. The reorganization into four scientific program areas and one administrative and supervisory Extramural Activities Program is illustrated below:


OFFICE OF THE DIRECTOR, NINCDS
COMMUNICATIVE DISORDERS
FUNDAMENTAL NEUROSCIENCES
EXTRAMURAL ACTIVITIES PROGRAM
NEUROLOGICAL DISORDERS
STROKE AND TRAUMA
SCIENTIFIC EVALUATION BRANCH

Members of the professional and secretarial staff of the former Regular Programs Branch and the Special Programs Branch have been distributed among the four program areas and have been combined with staff from the former Collaborative and Field Research Program who worked primarily with contracts. Thus, the Director of each Program theoretically may use either grants or contracts within wide limits to accomplish the objectives of his Program. The question of whether research training awards are to be fully interdigitated into this arrangement is still under consideration. They have been coded by Program Area so they may be included as part of the total effort of the appropriate Program. However, the management and administration of research training awards is so different from that for all other types of awards and the amounts of funds involved are presently so modest, that it seems doubtful whether it would be advantageous to fully integrate them with grants and contracts from the standpoint of grant administration and management.

The Extramural Activities Program provides supervisory, coordinating and service functions for the other Programs as well as for extramural activities in general. The top staff consist of:

Director
Deputy Director (Research Grants)
Assistant Director for Manpower Programs
Assistant Director for Contract Research

The responsibilities of these people are apparent from their titles in that they carry out an overall coordinating and supervisory function in regard to the implementation of recommendations of the NANCDS Council and Contract Advisory Committees, and the processing and issuance of proposals and awards in their respective areas. The Director, in consultation with the Director

NINCDS, in addition works closely with the other Program Directors on questions of policy, distribution of funds, etc. Each of these people have a summary of their activities elsewhere in this Report.

The Extramural Activities Program also includes a Grants Management Branch, a Contracts Management Branch and an Office of Data Analysis and Reports, each of which has its own summary of activities elsewhere in this Report.

Also included is a Scientific Evaluation Branch with the responsibility for the initial review of all grant and contract proposals which are not reviewed by Study Sections of the Division of Research Grants, an activity of the Office of the Director, NIH. This Branch is presently composed of five professional personnel and supporting staff including the Executive Secretaries of the Communicative Disorders Program Project Committee, the Neurological Disorders Program Project Committee A and the Neurological Disorders Program Project Committee B. The establishment of this Branch makes it possible, in agreement with NIH policy, to have a true dual review system for grant applications and contract proposals in which the initial review is carried out by Committees within the Institute. Previously the Executive Secretaries usually administered and managed grants which were reviewed by their own Committees. This responsibility has now been taken over by the staff of the Program to which the proposal is assigned. This Branch also has a summary of its activities elsewhere in this Report.

Last, but not least, is the Office of Program Support and Awards (grants and contract processing). This group prepares, coordinates and assembles all of the material for the final review of all grants and contracts. It sees that copies of proposals are distributed to appropriate staff members and it assembles 61 sets of books for each National Advisory Council meeting, each set consisting of three books totalling some 500 pages of initial review group recommendations. It also assembles similar books of material for the Council Planning Subcommittee which meets about two weeks before each Council meeting.

The rationale for the reorganization is that the Director of each Scientific Program should have available all of the mechanisms (grants, contracts, training awards) and he should use them more or less at his discretion to best accomplish the objectives of his Program. It is further felt that the Program Director, in consultation with the Advisory Committee which is being established for each Program, should exert a fair degree of control and direction on the program to best meet its specific objectives. It was decided that this arrangement would function most effectively if the grants activities and the contract activities were housed together. Therefore, the grants staff were moved from the Westwood Building to the Federal Building in March, 1976.

The problems which have arisen are exactly those which have been faced by all other Institutes organized in this way. The fundamental problem is to avoid having the extramural effort of the Institute break up into four separate and distinct programs, each going its independent way scientifically and administratively. It is well known that research in one area is frequently applicable to problems in another area. Therefore, it is essential that the Programs be interrelated through staff discussions and adequate liaison.

In general, rather specific allocations of funds are made to each Program. In the case of grants, the Program Directors have agreed that the best proposals above a certain priority cut-off should be supported regardless of what their relevance to a particular Program appears to be at the moment. An effort is made to reserve a modest amount of funds which the Program Directors may use to support other approved projects which they feel are especially important to their Program.

In summary the reorganization and the move were based on the philosophy that:

1. A Program Director should exercise an appreciable degree of direction and control of his Program.

2. This can be best accomplished by each Program Director having available at least the two main mechanisms of project support (grants and contracts) which should be utilized more or less interchangeably to attain the objectives of the Program.

3. The joint utilization of grants and contracts would be most effective if the respective staffs are housed in close proximity.

The primary logistical problems have been:

1. Attendance at NINCDS and NIH meetings, practically all of which are outside of the Federal Building, and involve transportation and parking problems.

2. Delays in receiving materials such as applications and summary statements which affect all of us, especially the Office of Program Support and Awards (OPSA) and

3. Limitations on office and working space which have been particularly severe for OPSA, the Scientific Evaluation Branch (SEB), and the Office of Data Analysis and Reports (ODAR).

Attendance at meetings at NIH is even more difficult than it was due to greatly restricted parking facilities as a result of the construction of the Ambulatory Care Center. There has been some improvement in the receipt of materials from the Westwood Building, Building 31, etc., as people have at least become aware of the correct address. However, there seems to be no way to avoid the fact that it takes three-four days to get applications and summary statements from DRG in the Westwood Building. Space problems have been ameliorated by the allocation of some more space to EAP as a result of the Health Resources Administration moving out of the Federal Building. Most of the additional 1,200 square feet will be divided among OPSA, SEB, and ODAR. One difficulty is that the new space is on the sixth floor. In order to have sufficient contiguous space for collating, etc., half of OPSA space will be on the 10th floor and half on the sixth floor. This obviously is quite inconvenient and inefficient.

One of the primary technical problems concerns the coding of competing grant applications for Program Areas by ODAR. Under the new schedule of deadlines, Study Section and Council meetings, the applications for the next following Council meeting (September) are received just before the current (May) Council meeting, a time when ODAR is especially busy with priority lists and other Council activities. Incoming applications must be arranged on the shelves numerically within each Program Area. Therefore, they must be coded by Program Area before they can be arranged. However, the coding by ODAR comes right at a time when ODAR is overwhelmed with work for the immediate Council meeting. As a result the coding, and the ready availability of applications to Program staff may be delayed, perhaps even until after Study Section meetings which now come at almost the same time as Council meetings. Like many other problems, this one results primarily from the very cyclic nature of many EAP activities. Consideration is being given to some type of reorganization within ODAR to resolve this problem.

As a matter of record, Table I is attached which shows the number of awards and the amounts of funds expended for each type of award within each Program Area.

TABLE I

Number of Awards and Dollars Expended by Program Area and Type of Award

PROGRAM AREAS

TYPE OF AWARD	CD		FN		ND		ST		TOTAL	
Research Grants	No. 252	$15.631	No. 373	$20.990	No. 490	$32.833	No. 157	$ 9.424	No. 1272	$78.878
Program Projects and Clinical Centers	No. 25	$ 8.376	No. 9	$ 3.108	No. 38	$15.967	No. 40	$15.886	No. 112	$43.337
Contracts	No. 11	$ .907	No. 18	$ 2.166	No. 24	$ 5.555	No. 18	$ 1.445	No. 71	$10.073
Old Training Grants	No. -	$ -	No. -	$ -	No. -	$ -	No. -	$ -	No. -	$ -
New Training Grants	No. 10	$ .911	No. 5	$ .445	No. 13	$ 1.181	No. -	$ -	No. 28	$ 2.537
New Fellowships	No. 51	$ .679	No. 38	$ .516	No. 282	$ 3.904	No. 11	$ .145	No. 382	$ 5.244
Teacher-Investigator Awards	No. 4	$ .106	No. -	$ -	No. 16	$ .387	No. 1	$ .037	No. 21	$ .530
Research Career Development Awards	No. 11	$ .350	No. 21	$ .644	No. 51	$ 1.605	No. 6	$ .187	No. 89	$ 2.786
Research Career Awards	No. 1	$ .033	No. -	$ -	No. 5	$ .160	No. 1	$ .031	No. 7	$ .224
TOTALS	No. 365	$26.993	No. 464	$27.869	No. 919	$61.592	No. 234	$27.155	No. 1982	$143.609

(Dollars in millions)

July 1, 1976 - September 30, 1977

ANNUAL REPORT
July 1, 1976 - September 30, 1977
Contract Research Programs
Extramural Activities
National Institute of Neurological and
Communicative Disorders and Stroke
National Institutes of Health

Research contracts are employed by the Institute when areas of research have been identified which are not receiving sufficient investigative attention, and when Institute staff are prepared to provide the necessary central direction, and management required by the contract mechanism. Contracts continue to account for about seven to eight percent of the Institute's budget.

Four of the Institute's six programs now have panels for review of contract project concepts and the remaining two programs will name such panels in the near future. Important steps have been taken in the effort to space contract projects throughout the fiscal year. At least three current competitive solicitations will result in contracts awarded during the early part of FY 1978. Also, several large contracts formerly expiring in June have had their expiration dates shifted into other months.

By the end of the fiscal year the NINCDS Contract Review Board will have assigned dollar amounts for future commitments for all contracts receiving technical merit review this year. A new version of the Contract Information System Printout makes this information readily available to Institute staff.

The handling of unsolicited proposals has been streamlined by allowing program areas only 30 days to arrive at a determination of interest in a specific proposal. This deadline has allowed the Institute to respond to offerors in a timely manner.

During the year, this office was established as the NINCDS focal point for the P.L.-480 Special Foreign Currency Program. Details on the current status of this program are to be found elsewhere in this report.

The expected number and dollar value of research contracts and agreements utilizing NINCDS contract funds for the period covered by this report are as follows:

	NUMBER	$ (IN THOUSANDS)
Communicative Disorders Program	14	907
Fundamental Neurosciences Program	17	2,165
Neurological Disorders Program	24	5,493
Stroke and Neural Trauma Program	20	1,528
Office of Biometry and Epidemiology	11	475
Intramural Research Program	28	2,981
	114	13,549

NINCDS
SPECIAL REPORT
Special Foreign Currency Program (P.L.-480)
Fiscal Year 1977

The NIH Special Foreign Currency Program can support collaborative research projects in countries where the U.S. Treasury Department has a balance of local currency excess to the needs of the U.S. At present, these countries are:

- Burma (For political and economic reasons, projects cannot be initiated unless some dollars are provided.)
- Egypt
- Guinea
- India
- Pakistan
- Poland (Through the U.S.-Polish Joint Committee for Cooperation in the Field of Health for support from the Marie Sklodowska-Curie Joint Fund.)

Recent bilateral agreements between the U.S. and Egypt, India, Israel, Poland, and Yugoslavia have provided previously non-existent official recognition of this program. Although priority areas of mutual interest have been developed as part of these agreements, there are provisions for consideration of any meritorious proposal which does not conform to the established priorities.

There is no additional U.S.-owned local currency available for projects in Israel or Yugoslavia. The bilateral agreements provide the following alternatives for support. An agreement with Israel established the U.S.-Israel Binational Science Foundation, which operates independently of NIH. Deposits of equal amounts by each country created a fund from which approximately $2.1 million equivalent accrues annually. Applications are submitted to the Executive Director of the Foundation in Jerusalem. Proposed projects must have a U.S. scientist identified as collaborator. After technical merit review, the Foundation's Board of Governors selects the projects to be funded.

The U.S.-Yugoslavia bilateral agreement has a unique provision for matching U.S. or NIH dollar support of any former SFCP or new research project of mutual interest on a 50-50 matching basis through a Joint Fund administered by the U.S.-Yugoslavia Joint Board of Scientific and Technological Cooperation. The Joint Fund was originally established for the purpose of providing matching dinar support during the phase-out period of the SFCP in Yugoslavia.

In Fiscal Year 1977, NINCDS had 19 ongoing projects. There were 2 projects in Egypt, 4 in India, 4 in Poland, and 9 in Yugoslavia. The scientific content of the P.L.-480 research is complementary to other NINCDS research and covers both clinical and laboratory studies. Each of the P.L.-480 projects must have a U.S. scientist to serve as a collaborating "Project Officer" who works with the foreign scientist in varying degrees. The role of the U.S. Project Officer varies from that of an active collaborator participating in the research to that of an informed sponsor. However, the Project Officer must each year review the expenditures and the progress report and advise the Institute whether the progress has been satisfactory. Of the 19 projects, 5 have Project Officers who are in the NINCDS. The other 14 Project Officers are in Universities throughout the country.

The following projects were active in FY 1977:

COUNTRY	PROJECT TITLE	PRINCIPAL INVESTIGATOR	PROJECT OFFICER
Egypt 03-006-N	Venoms of Poisonous Snakes	Dr. Ahmed M. Mohamed Faculty of Medicine Ain Shams University Cairo, Egypt	Dr. Richard L. Irwin NINCDS
Egypt 03-028-N	Biochemistry of Enzymes and Toxins in Snake Venoms	Dr. Farag Nagi Wahba Ain Shams University Cairo, Egypt	Dr. John H. Hash Vanderbilt University Nashville, TN
India 01-016-N	Neurologic Investigations of Cerebrovascular Disorders	Dr. Praful Dalal B.Y.L. Nair Charitable Hospital Bombay, India	Dr. R.D. Adams Dr. C.M. Fisher Massachusetts General Hospital Boston, MA
India 01-040-N	Polypeptide Constituents of Snake (Naja naja) Venoms	Dr. L.K. Ramachandran Osmania University Hyderabad, India	Dr. H. Fraenkel-Conrat University of Calif. Berkeley, CA
India 01-041-N	Effect of Protein Malnutrition on Brain Metabolism	Dr. Saroj Mehta Postgraduate Institute of Medical Education and Research Chandigarh, India	Dr. J.H. Menkes Veterans Administration Los Angeles, CA
India 01-125-N*	Stroke and Ischemic Heart Disease in India	Dr. C. Gopalan Indian Council of Medical Research New Delhi, India	Dr. Clark Millikan University of Utah College of Medicine Salt Lake, UT

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*Funding contingent on Indian Council of Medical Research accepting the recommendations of the NINCDS's technical merit review group.

COUNTRY	PROJECT TITLE	PRINCIPAL INVESTIGATOR	PROJECT OFFICER
Poland 05-002-N	Characteristics of Muscle Disease	Dr. Irena Hausmanowa-Petrusewicz Department of Neurology Medical Academy of Warsaw Warsaw, Poland	Dr. Lewis Rowland College of Physicians and Surgeons Columbia University New York, NY
Poland 05-013-N	Biology and Histochemistry of Gliomas	Dr. J. Alwasiak Pathology Institute Medical Academy Lodz, Poland	Dr. A.K. Ommaya NINCDS
Poland 05-083-N	Prostaglandin Fever Catecholamines Interaction	Dr. Jacek Splawinski Copernicus Medical Krakow, Poland	Dr. Eberhard Trams NINCDS
Poland 05-092-N	Biochemistry of Oligodendroglia, Meyelin and Demyelination	Dr. Mieczslaw Wender Medical Academy Poznan, Poland	Dr. Richard Quarles NINCDS
Yugoslavia 02-003-N	Neural Correlates of Behavior	Dr. Ljubisa Rakic Institute for Biology Academy of Sciences Belgrade, Yugoslavia	Dr. J.D. French University of Calif. Los Angeles, CA
Yugoslavia 02-006-N	Histogenesis of the Forebrain	Dr. V. Sulovic Multidisciplinary Center University of Belgrade Belgrade, Yugoslavia	Dr. R.L. Sidman Dr. P. Rakic Harvard Medical School Boston, MA
Yugoslavia 02-008-N	Function of Cholinesterases	Dr. M. Brzin University of Ljubljana Ljubljana, Yugoslavia	Dr. Melvin Yahr Mt. Sinai School of Medicine City University of NY New York, NY
Yugoslavia 02-015-N	Metabolism, Release, and Uptake of 5-Hydroxytryptamine	Dr. Zlatko Supek "Ruder Bosovic" Inst. Zagreb, Yugoslavia	Dr. F.M. Bumpus Cleveland Clinic Cleveland, OH
Yugoslavia 02-090-N	Gene Transcription and Expression	Dr. Vladimir R. Glisin Center of Multidisciplinary Studies Belgrade, Yugoslavia	Dr. Paul Doty Harvard University Cambridge, MA

COUNTRY	PROJECT TITLE	PRINCIPAL INVESTIGATOR	PROJECT OFFICER
Yugoslavia 02-023-N	Vestibular Influences on Cerebral Activity	Dr. Bosko Barac School of Medicine University of Zagreb Zagreb, Yugoslavia	Dr. G.H. Glaser Yale University New Haven, CT
Yugoslavia 02-025-N	Neuropathology of Closed Brain Injury	Dr. Nenad Grecevic University of Zagreb Zagreb, Yugoslavia	Dr. J. Kiffin Penry NINCDS
Yugoslavia 02-032-N	Sialidase Action on Complex Molecular Aggregates	Dr. Vaskresenija Lipovac University of Zagreb Zagreb, Yugoslavia	Dr. A. Rosenberg Pennsylvania State Univ. Hershey, PA
Yugoslavia 02-042-N	Brain Survival in Anoxia and Hypothermia	Dr. Radoslav K. Andjus Institute of Biological Research Belgrade, Yugoslavia	Dr. H.A. Sloviter Univ. of Pennsylvania Philadelphia, PA

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Extramural Activities
Manpower Programs
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

The Institute entered this reporting period after having fulfilled its obligations to all of the old training programs which had been in a phase-out status for about five years. Our support and efforts during this period were, therefore, devoted to the following four viable training programs: Individual National Research Service Award Program, Institutional National Research Service Award Program, Research Career Development Award Program, and Teacher-Investigator Award Program. The actual training appropriation for FY '77 was $8.1 million. This does not include transitional quarter support. Of this amount, $800,000 was transferred to research programs to assist in funding the Research Career Development Awards which, as of May 1, 1977, were awarded under new guidelines which increased the average award by approximately $10,000. This report concerns the specifics of these programs.

Individual National Research Service Award Program

This postdoctoral research training program receives the highest funding priority. After making what was considered an appropriate number of new Institutional National Research Service Awards, taking into consideration the increase in future years of committed support, the remainder of our training funds were used to support new applicants who submitted their own Individual NRSA applications and individuals who had committed years of support. During the five-quarter reporting period, we made the following number of new and continuation awards according to NINCDS program area:

Program	New Awards		Continuation Awards		Total	
	Number	Amount	Number	Amount	Number	Amount
Communicative Disorders	32	$443,394	19	$235,765	51	$679,159
Fundamental Neurosciences	13	254,300	25	261,700	38	516,000
Neurological Disorders	134	1,926,110	148	1,977,959	282	3,904,069
Stroke and Trauma	7	93,600	4	51,400	11	145,000

The above new awards includes about 75 awards which were recommended for funding by our National Advisory Council at its meeting in September 1977. These awards would normally have been funded out of FY '78 funds.

A major problem arose in this program for awardees who were being trained in government laboratories, including those at NIH. Their allowance for medical insurance and travel is restricted to $1,000 and, in the summer of 1977, the cost of the family plan for medical insurance increased to over $1,000. Thus trainees with families not only have to pay part of the medical insurance premium but have no funds for travel to professional meetings.

In discussing the funding of Individual NRSA applications in the future, NINCDS Program Directors agreed that awards would be made to individuals having applications with the highest merit ratings. Thus the cut-off for funding, according to priority level, would be the same for each program area. The funding of any application below the cut-off which was considered by a Program Director to be important to his program would be discussed individually with the Assistant Director for Manpower Programs.

Institutional National Research Service Award Program

From FY '77 funds, the Institute provided continuation support for 22 programs that had future years of committed support and made 14 new awards. No awards were made during the transitional quarter and FY '77 funds were used to support the following programs according to NINCDS program area:

Program	New Awards		Continuation Awards		Total	
	Number	Amount	Number	Amount	Number	Amount
Communicative Disorders	8	$229,285	7	$382,185	15	$911,470
Fundamental Neurosciences	3	281,815	2	163,255	5	445,077
Neurological Disorders	3	136,138	13	1,044,643	16	1,180,781

These 36 awards, for the year July 1, 1977 through June 30, 1978, provide stipend support for 164 individuals, 36 predoctoral trainees and 128 postdoctoral trainees.

In the future, the four NINCDS Program Directors will constitute an NINCDS Training Board and it will be the Board's responsibility to select the specific training programs to receive new or renewal support. The Board would, acting as a training subcommittee, review all applications and the Assistant Director for Manpower Programs would present the designated recommended applications to the National Advisory Council for funding.

The only receipt date for applications to be supported from FY '77 funds was April 1.

During the year, we had an opportunity to reconsider the areas in which we would make Institutional NRS Awards. In line with a recommendation of the National Research Council's study of Personnel Needs and Training for Biomedical and Behavioral Research, we made the training areas for the Individual NRSA Program and the Institutional NRSA Program identical. The announcement would read that both Individual and Institutional National Research Service Awards can be made in the following four areas with designated examples of specific areas in which applications would be appropriate:

1. Basic Neurosciences

    - Developmental Neurology
    - Neuroanatomy
    - Neurobiology
    - Neurochemistry
    - Neuroimmunology
    - Neuropharmacology
    - Neurophysiology
    - Neuroradiobiology
    - Neurovirology
    - Sensory Physiology and Biophysics

2. Clinical Neurosciences

    - Clinical Investigation
    - Neuroepidemiology
    - Neuropathology

3. Basic Communicative Sciences

    - Audiology
    - Sensory Physiology and Biophysics
    - Speech Pathology

4. Clinical Communicative Sciences

    - Audiology
    - Clinical Investigation
    - Otopathology
    - Speech Pathology

In addition to these areas, Institutional NRSA applications for training members of minority groups in the neurological and communicative sciences can be submitted in any area that would appropriate to the mission of the Institute.

When Institutional National Research Service Awards are made in the area of clinical investigation, some very specific guidelines have been drawn up to assist in the administration of the programs. The following guidelines were agreed upon by the four Program Directors:

1. No trainee may be appointed for less than 12 months.
2. At the time of appointment, the trainee must have completed at least two years of full-time clinical residency training if he is to be trained in clinical investigation. If the trainee is to study full-time in a basic area, he could become a trainee at any time.

3. No trainee may be appointed for more than 24 months of clinical research training.
4. The initial period of award for Institutional NRSA's is a maximum of three years.
5. The initial award will provide stipends for a maximum of two trainees for the first year and four trainees for the second and third years.

Research Career Development Award Program

During the year the NIH revised the guidelines governing the funding of RCDA applications. The maximum level of support was increased from $25,000 to $30,000 and, in addition, the NIH agreed to provide a proportionate share of the employer's contribution to fringe benefits and to provide 8% indirect costs.

During the five-quarter reporting period, we made the following number of new and continuation awards according to NINCDS program area:

Program	New Awards		Continuation Awards		Total	
	Number	Amount	Number	Amount	Number	Amount
Communicative Disorders	6	$176,100	5	$173,500	11	$349,600
Fundamental Neurosciences	4	123,500	17	520,600	21	644,100
Neurological Disorders	12	421,070	39	1,183,800	51	1,604,870
Stroke and Trauma	1	31,500	5	155,500	6	187,000

In the future, the four NINCDS Program Directors will constitute an NINCDS Training Board which will be responsible for selecting the specific RCDA applications to be funded. The Board, acting as a training subcommittee, would review all applications and the Assistant Director for Manpower Programs would present the designated recommended applications to the National Advisory Council for funding.

Teacher-Investigator Award Program

During this reporting period, the Institute made a major change in support for new Teacher-Investigator Awardees. Their support was made comparable to that of the RCD Awardees. Thus, instead of pegging the stipend to number of years of relevant postdoctoral research and/or training, it was made for the amount that would normally be paid to such individuals at the awardee institution, but not to exceed $30,000. In addition, the award includes a proportionate share of the employer's contribution to fringe benefits and 8% indirect costs.

During the five-quarter reporting period, we made the following number of new and continuation awards according to NINCDS program area:

Program	New Awards		Continuation Awards		Total	
	Number	Amount	Number	Amount	Number	Amount
Communicative Disorders	1	$26,000	3	$80,000	4	$106,000
Fundamental Neurosciences	-	--	-	--	-	--
Neurological Disorders	3	85,250	11	273,000	14	358,250
Stroke and Trauma	1	39,400	2	26,000	3	65,400

One Program Director elected to fund an appreciable number of Teacher-Investigator Applications from funds available to his program in FY '78. In the future, applications to be funded will be those recommended by the various program directors. These will be identified for the Assistant Director for Manpower Programs who will present them to the National Advisory Council as the applications which are being recommended for funding.

MANPOWER EVALUATION STUDIES

In 1972 and 1973, the Institute awarded contracts to study manpower needs in speech pathology and audiology, otolaryngology, neurology, neurological surgery, and the basic sciences related to neurology and the communicative areas.

The reports in speech pathology and audiology, otolaryngology, and neurology are in the process of being printed by the Government Printing Office. The neurological surgery report requires additional work to put it into shape. The final report on the basic sciences study has just been received from the contractor, the National Research Council, National Academy of Sciences. It too will ultimately be printed by the Government Printing Office.

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Contracts Management Branch
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

The Contracts Management Branch (CMB) consists of the Contracting Officer who is Chief of the Branch, three Contract Specialists and two supporting staff.

The CMB is responsible for the administration of some 114 contracts and Interagency Agreements totaling $13.5 Million. In addition there are 70 completed contracts in various stages of being closed administratively.

The CMB expects to award approximately 15 new contracts during FY 77. Added to this workload is 41 renewals of existing contracts and over 130 actions modifying contracts which do not involve additional funds.

During this reporting period the CMB conducted 5 seminars for contract project officers and a general one for Extramural Personnel. The Branch has noticed a marked appreciation of contract administration requirements as a result of these seminars.

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Extramural Activities
Grants Management Branch
National Institute of Neurological and Communicative Disorders and Stroke

Due to the greater budget restraints experienced this year, the Grants Management Branch has undergone considerable budget involvement with the program staff on a number of grants involving split funding. Each one of the Grants Management Specialists had responsibility for following up their program area with the program administrator and the grantees.

Our Grants Management Specialists keep an updated account of the Direct and Indirect Costs charged against each grant prior to awarding, indicating the charges to date against the Program Area.

Our Grant Close-Out Procedure has been experiencing some operational difficulty. Our grants technical assistant requires a listing of all terminating grants for each fiscal year. This enables our office to initiate action for the closeout procedure as well as a means of determining that we have carried out actions on all terminated grants. Listings furnished us by the Office of Data Analysis and Reports found to contain omissions have been called to their attention. They are working in conjunction with DRG to eliminate this deficiency.

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Scientific Evaluation Branch
National Institute of Neurological and Communicative Disorders and Stroke

The Scientific Evaluation Branch (SEB) was established in 1975 and assigned responsibility for the technical merit and scientific review of Contracts, Program Projects, Clinical Research Centers, Institutional Fellowships and Teacher-Investigator Awards. The Communicative Disorders Review Committee, Neurological Program Project A Committee, Neurological Program Project B Committee, and Special Review Committee are components of the SEB and provide the initial technical merit and scientific review of the proposals for the Institute. Summary statements are prepared by the SEB for the Institute staff, Contract Review Board and the Institute National Advisory Council.

The SEB has established liaison with the Intramural Program of the Institute, the Communicative Disorders Program, Fundamental Neuroscience Program, Neurological Disorders Program, and the Stroke and Trauma Program to facilitate the technical merit and scientific review of contracts, grants and fellowships. The SEB maintains a continuing liaison with leaders of the scientific community for the purpose of identifying the most qualified persons to serve on SEB panels and committees.

ANNUAL REPORT

July 1, 1976 to September 30, 1977
Extramural Activities Program
Data Analysis and Reports

National Institute of Neurological and Communicative
Disorders and Stroke

The Office of Data Analysis and Reports is fully operational for research and training grants, fellowship awards and institutional trainee appointments. The contracts, assigned to NINCDS-EA over a year ago, are nearly ready to be incorporated in this data base. The data base used now for managing contracts was never linked with the Division of Research Grants (DRG) contracts data for NINCDS. Several man months of editing and of arranging with DRG for certain types of retrieval means that NINCDS-EA will be able to rely in FY 1978 on the DRG contracts data base and the powerful programming retrieval capability which they offer.

The surge of reclassification workload resulting directly and indirectly from the Institute's reorganization has lightened briefly, but will increase as attention is focused on a new and much more intricate classification system to be operated in parallel with the existing system. ODAR has trained for, and plans continuing classification training in order to be partially staffed to meet this onerus possibility. Old and new systems (for contracts or for classification) need to be operated in parallel to insure comparability and thus confidence in the data's accuracy. Such comparability is also essential for trend comparisions.

Another major data abstracting, storage, edit, and retrieval task is also underway involving only program projects (P01's) and clinical centers (P50's) and their subprojects. NINCDS-EA manages some 90 such grants with a total of about 700 included subprojects, all of which needed to be abstracted and accounted for individually. Regular (R01) grants do not have subprojects and typically have awards that are no larger than an average P01 or P50 subproject.

These data are currently kept separately from a similar DRG data base, which should be modified appropriately for NINCDS-EA purposes before 1978. All of NIH will be converting to a Summary-Statement-retrieved multiprojects data system covering P01 and P50 grants in 1978. The NINCDS-EA base will be fully compatible but will possess expanded subproject budgets for about half of its grants when DRG obtains the first expanded subproject budget. Steps have been taken to develop interim update and retrieval programming for FY 1978 pending DRG development, to be available in late 1978 or early 1979.

ODAR prepares data books, fiscal year-end summary books, special reports and verbally presented fiscal trend reports to Program Area Directors or to Council, as well as a frequently updated listing of targeted research grants and of P01 and P50 grants with their subprojects. These categories of ODAR output have been changed and improved in FY 1977 imposing an increased workload. For example, a multiprojects listing for P01 and P50 grants is produced quarterly; special grants listings are more in demand to go to the four program areas. And program area classifications must be assigned quickly in order to deliver grant application to the program areas.

Among the new reports that will be available in FY 1978 will be a modified research grants and contracts index, containing only NINCDS grants and contracts classified by the NINCDS code and specially cross indexed.

Despite the loss of one person last December no unusual delays or data management problems are anticipated, assuming that continued administrative support is focused on assuring computer printing, courier services, xerox reducing and assuming that the revision and implementation of another NINCDS classification system will not impose a greatly increased workload.

New programming normally will be developed more slowly with this group's manpower shortage, and clerical backup will be needed at least on a part time basis for continuity of some services.

Table of Organization

Intramural Research Program

National Institute of Neurological and Communicative Disorders and Stroke

(Personnel on hand May 1977)

OFFICE OF THE DIRECTOR

Director, Intramural Research Program - Thomas N. Chase, M.D.
Laboratory Director, IRP - Richard L. Irwin, Ph.D.
Clinical Director, NINCDS - Donald B. Calne, M.D.
Medical Officer - Choh-Luh Li, M.D.
Administrative Officer - Glenn E. Hammond
Administrative Officer - Robert N. Knickerbocker
Administrative Assistant - Linda V. Christoferson
Writer Editor - Carol A. Feld
Psychologist - Susan M. Pollard
Psychologist - Andrea C. Gielen
Administrative Technician - Doris R. Perry
Travel Assistant - Ida M. Chernikoff
Procurement Assistant - Patricia D. Williams
Secretary (Steno) - Margaret Henry
Secretary (Steno) - Vernita Bergmeyer
Secretary (Steno) - Corinne C. Gillis
Secretary (Steno) - Doris Selkowitz
Budget Clerk (Typing) - Carolyn L. Peel
Clerk-Stenographer - Daryl Dawson
Clerk-Stenographer - Beth A. Garman
Clerk-Typing - Lillian L. Wease
Clerk-Typing - Ann T. Miller
Clerk-Typist - Nancy Snowden
Clerk-Typist - Dorothy Mudrick
Biologist - Minnie Toure
Biological Laboratory Technician (Animal) - George R. Duvall
Biological Laboratory Technician (Animal) - Adrian P. Loftis
Biological Laboratory Technician - Alexander Wheaton
Biological Laboratory Technician (Micro) - Charles E. Sartor
Medical Technician - Tsin Yeun Ling
Office Machine Operator - Kenneth M. Oglesbee
Laboratory Worker - John R. Bowers
Visiting Fellow - George Andrews, M.B.B.S.

Section on Technical Development

Computer Systems Analyst - William H. Sheriff, Jr.
Biomedical Engineer - Bruce M. Smith, Ph.D.
Staff Fellow - Susan Hauser, Ph.D.
Engineering Technician - Michael J. Walsh

NINCDS Guam Research Center

Health Technician - Jose M. Torres
Health Technician - Manuel T. Cruz
Health Technician - Francisco Leon-Guerrero
Nurse - Marjorie Gillespie
Biological Laboratory Technician (Micro) - Luiz T. Munoz
Secretary (Typing) - Mary E. Hernandez

MEDICAL NEUROLOGY BRANCH

Office of the Chief

Chief, Medical Neurology Branch - W. King Engel, M.D.
Assistant Neurologist - Roger W. Kula, M.D.
Medical Officer - John Richardson, M.D.
Medical Officer - Bruce T. Adornato, M.D.
Medical Officer - Roger A. Brumback, M.D.
Medical Officer - William J. Stump, M.D.
Medical Officer - Robert Shebert, M.D.
Visiting Scientist - Jaap Bethlem, M.D.
Visiting Associate - Tulio Bertorini, M.D.
Visiting Associate - Carlo Yuson, M.D.
Staff Fellow - Jan Kucera, M.D.
Biologist - Katherine Oliver
Biological Laboratory Technician (Micro) - Guy G. Cunningham
Microbiologist - Priscilla Chauvin
Physiologist - E. Carolyn Derrer
Biologist - Mary A. Oberc
Biologist - Gregory C. Zirzow
Biological Laboratory Technician - Samuel T. Carroll
Biological Laboratory Technician - Jane V. Lawrence
Biological Laboratory Technician - Greggory Hubbard
Histopathology Technician - Thelma Fletcher
Biological Laboratory Technician - Mitchell Burken
Secretary - Lucy L. Riley
Secretary (DMT) - Gertrude Wright
Secretary (DMT) - Emma P. Dick
Clerk (Typing) - Mary E. Mattia
Clerk (Typing) - Alberta L. Huff
Clerk-Typist - Beverly Skelton
Clerk-Typist - Michelle Clemons
Biological Aid - Margaret Vaughan

Biological Aid - Marshall Montrose
Biological Aid - Robert Vanderbilt
Biological Aid - Peter T. Ching
Biological Aid - Karita M. Parker
Biological Aid - Janet M. Harner
Biolgoical Aid - Dale C. Doerer
Laboratory Worker - Matthew P. Meadows
Visiting Fellow - Katerina Dorovini-Zis, M.D.

SURGICAL NEUROLOGY BRANCH

Office of the Chief

Acting Chief, Surgical Neurology Branch - Ayub K. Ommaya, F.R.C.S.
Visiting Associate - Mohammed Mendhi, M.D.
Secretary (Steno) - Olga G. Williams
Secretary (DMT) - Nelva L. Reckert

Applied Research in Surgical Neurology Section

Medical Officer - Bernard Frankel, M.D.
Histopathology Technician - Martha L. Johnson
Biological Laboratory Technician (Biochem) - M. Arthur Banks
Biological Laboratory Technician (Animal) - Calvin S. Hawkins
Biological Laboratory Technician (Animal) - Clifford A. Seay
Biological Aid - Stephen O'Donnell

Neuroradiology and Computed Tomography Section

Research Medical Officer - Giovanni Di Chiro, M.D.
Visiting Scientist - Leo Dubal, M.D.
Visiting Scientist - Tetsuo Arimitsu, M.D.
Sr. Staff Fellow - Mary K. Hammock, M.D.
Sr. Staff Fellow - Rodney A. Brooks, M.D.
Medical Radiology Technician - Jane W. Dove
Clerk-Typist - M. Jacqueline Pattison
Clerk-Typist - Frances A. Harris
Biological Aid - David Handelsman

CLINICAL NEUROSCIENCES BRANCH

Office of the Chief

Chief, Clinical Neurosciences Branch - Cosimo Ajmone Marsan, M.D.
Secretary (DMT) - Tina McDaniel
Clerk-Stenographer - Mary Vassiliades
Clerk-Typist - Sheila Stokes

Clinical Neurophysiology Section

Medical Officer - Nick Olmos-Lau, M.D.
Medical Officer - Donald W. King, M.D.
Medical Officer - James B. Macon, M.D.
EEG Technician - Joan Trettau
Biologist - Stuart Walbridge
EEG Technician - Martha H. Fair

Functional Neurosurgery Section

Associate Chief, Clinical Neurosciences Branch - John M. Van Buren, M.D.
Research Psychologist (Clinical) - Paul Fedio, Jr., Ph.D.
Biologist - Rosemary C. Borke
Psychologist - Ghislaine R. Frederick
Histopathology Technician - Vivian A. Betton
Clinical Psychologist - John Oubre
Clerk-Stenographer - Michele Shevitz
Biological Aid - Keith Reid

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH

Office of the Chief

Chief, Developmental & Metabolic Neurology Branch - Roscoe O. Brady, M.D.
Secretary (Steno) - Barbara W. Critzer
Clerk (DMT) - Lois A. Brown
Clerk-Typist - Sheila Y. Burris
Visiting Fellow - Rosemary McIntyre, Ph.D.

Enzymology and Genetics Section

Research Chemist - Richard Quarles, Ph.D.
Research Chemist (Biochem) - Peter H. Fishman, Ph.D.
Research Chemist - Peter G. Pentchev, Ph.D.
Medical Officer - Daniel E. Britton, M.D.
Medical Officer - John A. Barranger, M.D.
Sr. Staff Fellow - F. Scott Furbish, Ph.D.
Staff Fellow - John W. Kusiak, Ph.D.
Chemist - Roy M. Bradley
Chemist - Jane M. Quirk
Biological Laboratory Technician (Biochem) - George E. Mook
Visiting Fellow - Laurence McIntyre, Ph.D.
Visiting Fellow - Tadeusz Pacuszka, Ph.D.

Neurochemical Methodology Section

Research Chemist - Andrew E. Gal, Ph.D.
Biological Laboratory Technician (Biochem) - Frank J. Fash

Clinical Investigation and Therapeutics Section

Assoc. Chief, Developmental & Metabolic Neuro. - Anatole Dekaban, M.D.
Research Chemist - George Constantopoulos, Ph.D.
Medical Technician (Chem.) - Jan K. Steusing

Physiology Metabolism Section

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter
Biological Aid - Linda A. Garrett

EXPERIMENTAL THERAPEUTICS BRANCH

Office of the Chief

Chief, Experimental Therapeutics Branch - Donald B. Calne, M.D.
Clerk (Typing) - Elizabeth Bliss
Visiting Fellow - Christopher Aylmer, Ph.D.

Therapeutics Section

Visiting Associate - Paul F. Teychenne, M.D.
Visiting Associate - Adrian Williams, M.D.
Sr. Staff Fellow - Judith R. Walters, Ph.D.
Histopathology Technician - Raymond R. Vane
Biologist - Barbara H. Byse
Biologist - Joan M. Lakoski
Clerk (DMT) - Pamela Barnicoat
Biological Aid - Charles Plotkin
Biological Aid - Shawn W. Kennedy

Pharmacology Section

Medical Officer - William Lightfoote, M.D.
Medical Officer - John G. Nutt, M.D.
Staff Fellow - Ellen Silbergeld, Ph.D.
Chemist - Nancy Eng
Secretary (Typing) - Sandra M. Meadows
Biological Aid - Hilary S. Adler
Biological Aid - Reeda L. Harris

Clinical Epilepsy Section

Sr. Staff Fellow - John W. Kebabian, Ph.D.
Staff Fellow - Leonard P. Miller, Ph.D.
Clerk-Stenographer - Myrtle Sullivan

NEUROIMMUNOLOGY BRANCH

Office of the Chief

Chief, Neuroimmunology Branch - Dale E. McFarlin, M.D.
Medical Officer - Henry McFarland, M.D.
Medical Officer - Leslie B. Barnett, M.D.
Staff Fellow - Joseph F. Poduslo, Ph.D.
Staff Fellow - Eugene D. Johnson, Sc.D.
Staff Fellow - William Bellini, Ph.D.
Chemist - Elizabeth Mingioli
Medical Technologist - Carolyn A. Pedone
Biological Laboratory Technician - Toby Behar
Chemist - Peggy Weston
Secretary (Steno) - Shirley Sutphin
Clerk-Typist - Sheila Smallberg
Clerk-Typist - Barbara Shryock
Clerk-Typist - Donna S. Walters
Biological Aid - Andrew F. Carlson
Psychology Aid - Nancy Tang
Biological Aid - Jody M. Wildy
Visiting Fellow - Alan G. Trudgett, Ph.D.

INFECTIOUS DISEASES BRANCH

Office of the Chief

Chief, Infectious Diseases Branch - John L. Sever, M.D.
Secretary (Steno) - Ethel M. Ingram
Procurement Clerk - Florence Reid
Clerk-Typist - Sharon K. Painter
Clerk-Stenographer - Norma Graber

Experimental Pathology Section

Veterinarian - William T. London, D.V.M.
Research Veterinarian - Amos E. Palmer, D.V.M.
Biologist - Blanche Curfman
Biological Laboratory Technician (Animal) - Robert L. Brown
Biological Laboratory Technician (General) Geneva M. Brown
Biological Laboratory Technician (Animal) - Joseph Ricketts
Biological Aid (Animal) Wayne E. Nusbaum
Biological Aid - Sherry L. Thomas
Animal Caretaker - Adley J. Atkinson
Laboratory Worker - Carl Rollison
Laboratory Worker - Harry Baker
Laboratory Worker - Mary Dorsett
Laboratory Worker - Claude T. Belcher

Immunochemistry and Clinical Investigations Section

Veterinarian - David L. Madden, D.V.M.
Medical Officer - Sidney A. Houff, M.D.
Microbiologist - Anita C. Ley
Nurse - Dorothy Edmonds
Biological Laboratory Technician - Paul P. Becher
Biological Laboratory Technician - Gene M. Brashears
Biological Laboratory Technician - Linda J. Perry
Medical Technician - Ampar Strickland
Biological Laboratory Technician (Micro) - Nancy R. Tzan
Biological Aid - Leonard Moore
Clerk - Frederick Brownholtz
Visiting Fellow - Morag Ferguson, Ph.D.

Neurovirology Section

Research Microbiologist - Maneth Gravell, Ph.D.
Visiting Scientist - J. Woychiechowska, Ph.D.
Sr. Staff Fellow - William C. Wallen, Ph.D.
Microbiologist - Flora Moder
Microbiologist - Renee G. Traub
Nurse Specialist - Helen M. Krebs
Biologist - Rebecca Hamilton
Biological Laboratory Technician (Micro) - Aurellia S. Krezlewicz
Biologist - Kenneth Rich
Biological Laboratory Technician - Frank J. West
Microbiologist - Mary A. Krasny
Biologist - Raymond H. Kiefer
Biological Laboratory Technician (Micro) - Janet M. Mattson
Microbiologist - Isabel Shekarchi
Microbiologist - Otto Gutenson
Biological Laboratory Technician - Ann P. Claysmith
Biological Aid - Mitchell Binder
Biological Aid (Biochem) - Kenneth A. Blank
Biological Aid - Joseph D. Murray
Laboratory Worker - Douglas Sykes

Neurogenetics Section

Medical Officer - Roswell Eldridge, M.D.
Technical Research Assistant - Thelma Koerber
Clerk-Typist - Valerie Washington
Clerk-Typist (trainee) - Andrew May

Electron Microscopy Section

Visiting Scientist - Monique Dubois, Ph.D.
Biologist - Edna Worthington
Biological Aid - Jeffrey B. Tate
Visiting Fellow - Marcos Rodriguez, Ph.D.

Visiting Fellow - E. Hooghe-Peters, Ph.D.
Visiting Fellow - C. Pottelsberghe, Ph.D.
Visiting Fellow - Bernard Rentier, Ph.D.

LABORATORY OF EXPERIMENTAL NEUROLOGY

Chief, Experimental Neuolrogy - William Caveness, M.D.
Visiting Scientist - Motohiro Kato, M.D.
Visiting Associate - Shinich Wakisaka, M.D.
Chemist - Raymond R. O'Neill
EEG Technician - Barbara Malamut
Secretary (Typing) - Marguerite Smiley
Visiting Fellow - Shinich Hosokawa, M.D.
Visiting Fellow - H. Pockberger, Ph.D.

LABORATORY OF NEUROPATHOLOGY AND NEUROANATOMICAL SCIENCES

Office of the Chief

Chief, Neuropathology & Neuroanatomical Sciences - Igor Klatzo, M.D.
Editorial Assistant - Jane T. Phelps
Biological Laboratory Technician (Animal) - Willie Perkins
Biological Laboratory Technician (Animal) - Albert V. Cantu
Biological Laboratory Technician (Animal) - Melvin H. Carroll
Clerk (Typing) - S. Joan Eskin
Visiting Fellow - Keiji Nishimoto, M.D.

Functional Neuroanatomy Section

Medical Officer - Thomas S. Reese, M.D.
Biological Laboratory Technician (General) - Frank D. Nolan
Biologist - Kathryn J. Lynch

Neurocytology Section

Research Physiologist - Milton Brightman, Ph.D.
Staff Fellow - Richard Broadwell, Ph.D.
Histopathology Technician - Gertrude Goping
Biologist - Barbara Reese
Biological Aid - Janice Harrison

Experimental Neuropathology Section

Medical Officer - Jan Cammermeyer, M.D.
Biologist - Iris Fenton
Biological Aid - Sophia Grabinski

Cerebrovascular Pathology Section

Visiting Scientist - Mieczysl Smialek, M.D.
Biological Laboratory Technician - Joseph T. Walker

Cellular Neuropathology Section

Assoc. Chief, LNNS - Henry DeF. Webster, M.D.
Staff Fellow - Michael J. Cullen, Ph.D.
Biological Laboratory Technician - Maureen O'Connell
Histopathology Technician - Kathryn Winchell
Biological Aid - Susan Larrick
Biological Aid - Linda M. Hanks
Visiting Fellow - Takeshi Tabira, M.D.

Neurocytobiology Section

Medical Officer - Maria Spatz, M.D.
Research Biologist - Margaret Ledoux, Ph.D.
Visiting Associate - Moshe Wolman, M.D.
Biological Laboratory Technician (Micro) - Madora E. Swink
Biologist - Joliet Y. Bembry
Biological Aid - Barbara D. Minor

LABORATORY OF NEUROPHYSIOLOGY

Office of the Chief

Chief, Laboratory of Neurophysiology - Michelango Fuortes, M.D.
Visiting Associate - Robert McBurney, Ph.D.
Biological Laboratory Technician (Animal) - William L. Beane
Clerk (DMT) - Gloria K. Hall
Clerk-Stenographer - Mary Lou Adams

General Physiology Section

Sr. Staff Fellow - Richard Normann, Ph.D.
Visiting Fellow - Jiri Pochobradsky, Ph.D.

Sensory Physiology Section

Medical Officer - Thomas G. Smith, Jr., M.D.
Research Associate - Jeffrey Barker, M.D.
Staff Fellow - Kin Futamachi, Ph.D.
Biomedical Engineer - Judith A. Resnik

Cell Biology Section

Research Biologist - Arnaldo Lasansky, M.D.
Visiting Associate - Silvana Vallerga, Ph.D.
Biological Laboratory Technician - Julia M. Lohr
Visiting Fellow - Menachem Hanani, Ph.D.
Visiting Fellow - Ido Perlman, Ph.D.

Neuronal Interactions Section

Head, Neuronal Interactions Section - Henry G. Wagner, M.D.
Sr. Staff Fellow - Helga E. Stanbury, Ph.D.

LABORATORY OF BIOPHYSICS

Office of the Chief

Chief, Laboratory of Biophysics - William J. Adelman, Jr., Ph.D.
Clerk (Typing) - Hazel W. Fermino
Visiting Fellow - Yoram Grossman, Ph.D.

Neural Membranes Section

Research Biophysicist - Kenneth S. Cole, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Visiting Associate - Robert J. French, Ph.D.

Neural Systems Section

Research Medical Officer - Daniel L. Alkon, M.D.
Visiting Associate - Tadashi Akaike, M.D.
Staff Fellow - Eliahu Heldman, M.D.
Visiting Fellow - I. Lederhendler, Ph.D.

Molecular Biophysics Section

Acting Head, Molecular Biophysics Section - Gerald Ehrenstein, Ph.D.
Supervisory Research Physiologist - Robert E. Taylor, Ph.D.
Research Physicist - Richard Fitzhugh, Ph.D.
Research Physiologist - Daniel L. Gilbert, Ph.D.
Electrical Engineer - Leonard Binstock
Research Physicist - Harold Lecar, Ph.D.
Mathematician - John Shaw
Electronic Technician - Herbert Walters
Secretary (Steno) - Maxine Schaefer
Biological Aid - Sandra I. Dixon
Biological Aid - Helen C. Fliegel
Biological Aid - John P. Irey
Biological Aid - Norman Magid

LABORATORY OF NEUROCHEMISTRY

Office of the Chief

Acting as Chief, Lab of Neurochemistry - Janet Passonneau, Ph.D.
Research Associate - Lawrence Rodichok, M.D.
Clerk-Stenographer - Sylvia Levinson
Clerk-Stenographer - Judy A. Zangardi
Clerk-Typist - Jacqueline Childs
Visiting Fellow - Nobaru Murakami, M.D.

Enzyme Chemistry Section

Research Chemist - R. Wayne Albers, Ph.D.
Visiting Associate - N. Krishnan, Ph.D.
Staff Fellow - Stephen Goldman, Ph.D.
Staff Fellow - Ann S. Hobbs, Ph.D.
Chemist - Oscar M. Young
Physical Science Technician (Chem.) - Eunice L. Summers

Neuronal Development and Regeneration Section

Medical Officer - Andrew Zalewski, M.D.
Health Services Officer - George Creswell
Biological Laboratory Technician (Micro) - Janina D. Ziemnowicz

Cellular Neurochemistry Section

Research Pharmacologist - Wesley D. Lust, Ph.D.
Staff Fellow - David McCandless, Ph.D.
Chemist - Gretchen Feussner
Biological Aid - Karen Wayns

LABORATORY OF MOLECULAR BIOLOGY

Office of the Chief

Chief, Laboratory of Molecular Biology - Ernst Freese, Ph.D.
Visiting Associate - Juan M. Lopez, M.D.
Staff Fellow - Peter Cooney, Ph.D.
Staff Fellow - Kenneth E. Rich, Ph.D.
Secretary (Steno) - Geraldine Bitango
Computer Aid (Typing) - Donna L. Hanyo
Clerk-Typist - Mary L. Keplinger
Visiting Fellow - Albrecht Bruckner, Ph.D.
Visiting Fellow - Patricia Whiteman, Ph.D.
Visiting Fellow - Ayub K. Yousufi, Ph.D.
Visiting Fellow - Takahiko Mitani, Ph.D.
Visiting Fellow - N. Vansantha, Ph.D.

Developmental Biology Section

Research Biologist - Elisabeth Freese, Ph.D.
Visiting Associate - Robert A. Pearce, Ph.D.
Medical Officer - Richard Henneberry, Ph.D.
Staff Fellow - Barbara C. Levin, Ph.D.
Biological Laboratory Technician - Norma Slepecky
Biological Aid - Abraham Rosner
Biological Aid - Debra A. Hlavaty
Visiting Fellow - Yasutaro Fujita, Ph.D.
Visiting Fellow - Kazuhito Watabe, Ph.D.

Molecular Virology Section

Research Chemist - Robert Lazzarini, Ph.D.
Staff Fellow - Jack Keene, Ph.D.
Staff Fellow - Marie L. Dirksen, Ph.D.
Staff Fellow - David A. Epstein, D.S.C.
Chemist - Leslye E. Johnson
Biological Aid - Stephen I. Adler
Biological Aid - John J. McNerney
Laboratory Worker - Margaret L. Shea
Laboratory Worker - Roberta E. Weaver
Laboratory Worker - Fritz E. Gerald
Laboratory Worker - Ernest C. Harr, Jr.
Visiting Fellow - Toshiro Adachi, Ph.D.
Visiting Fellow - Georgin Faulkner, Ph.D.

LABORATORY OF PERINATAL PHYSIOLOGY

Office of the Chief

Chief, Laboratory of Perinatal Physiology - Ronald E. Myers, M.D.
Research Psychologist - Shun-ichi Yamaguchi, Ph.D.
Biological Laboratory Technician (Animal) - William Rodriguez
Chemist (Biochem) - Carmen L. Freixas
Clerk (DMT) - Phyllis Goldberg

LABORATORY OF NEURAL CONTROL

Office of the Chief

Chief, Laboratory of Neural Control - Robert E. Burke, M.D.
Biological Engineer - Edward M. Schmidt
Research Physiologist - William B. Marks
Medical Officer - Gerald E. Loeb, M.D.
Engineering Technician - George M. Dold
Electronics Engineer - Martin J. Bak

Physiologist - Joan S. McIntosh
Clerk-Stenographer - Joan E. Kraft
Laboratory Worker - Danessa Drumgold
Visiting Fellow - Jacques Duysens, Ph.D.
Visiting Fellow - Bruce Walmsley, Ph.D.
Visiting Fellow - John A. Hodgson, B.S.C.

LABORATORY OF NEURO-OTOLARYNGOLOGY

Office of the Chief

Chief, Laboratory of Neuro-Otolaryngology - Jorgen Fex, M.D.
Sr. Staff Fellow - Dennis Drescher, Ph.D.
Staff Fellow - Joe C. Adams, Ph.D.
Staff Fellow - Robert Wenthold, Ph.D.
Biologist - Gabrielle Neises
Electronics Engineer - William Livingston
Chemist - Kang Sun Lee
Electronics Technician - Michael J. Frye
Chemist - Susanna W. Jones
Histopathology Technician - Marianne Parakkal
Secretary (Steno) - Elsie Walter
Clerk-Typist - Doreen Dalessandro

LABORATORY OF CENTRAL NERVOUS SYSTEM STUDIES

Office of the Chief

Chief Lab of Central Nervous System Studies - D. Carleton Gajdusek, M.D.
Supervisory Research Microbiologist - Clarence J. Gibbs, Ph.D.
Medical Officer - David M. Asher, M.D.
Medical Officer - Paul W. Brown, M.D.
Medical Officer - Lon R. White, M.D.
Veterinarian - Herbert L. Amyx, D.V.M.
Sr. Staff Fellow - George J. Nemo, Ph.D.
Staff Fellow - Ralph M. Garruto, Ph.D.
Staff Fellow - Robert G. Rohwer, Ph.D.
Biological Laboratory Technician - Michael P. Sulima
Biological Laboratory Technician - Alfred E. Bacote
Biological Laboratory Technician - John L. Priester
Microbiologist - Nancy P. Luber
Social Science Analyst - Judith B. Meyer
Anthropologist - Laura A. Kreiss
Anthropologist - Richard Benfante
Biological Laboratory Technician - Helena L. Gilbert
Mathematician - Steven G. Ono
Microbiologist - Randolph Taylor
Secretary (Steno) - Marion F. Poms

Secretary (DMT) - N. LaDonna Tavel
Clerk (DMT) - Wendy W. Showers
Translator - Jose Figirliyong
Biological Laboratory Technician - Diane M. Gifford
Biological Laboratory Technician - Gerald M. Murphy
Biological Aid - Judith P. Chavis
Biolgoical Aid - Diana B. Haydu
Biological Aid - Debra Smith
Biological Aid - Sylvia Anderson
Biological Aid - Thomas E. Curry
Biological Aid - Richard R. Jones
Biological Aid - Linda V. Springs
Health Technician - Ivan M'Baginatao
Animal Caretaker - Hubert O. Saville
Animal Caretaker - Ezra Shafer
Animal Caretaker - Harry Winpigler
Animal Caretaker - Leon J. Vance
Animal Caretaker - Eugene D. Webster
Visiting Fellow - Hiroshi Oda, M.D.
Visiting Fellow - Marie C. Mareau, Ph.D.

Annual Report of the Scientific Director
# of the
National Institute of Neurological and
Communicative Disorders and Stroke
July 1, 1976 through September 30, 1977

The Intramural Research Program of the National Institute of Neurological and Communicative Disorders and Stroke conducts research related to the nervous system and its disorders at the NIH Bethesda campus, as well as at Ft. Detrick, Maryland; Woods Hole, Massachusetts; and the Guam Research Center. To develop new knowledge essential to the prevention and treatment of nervous system disease, the Intramural Program has built a unique investigative capability which now embraces nearly every aspect of basic and clinically applied neurosciences research. Through direct operation of its own laboratories and clinics as well as through supervision of research contracts, the Program has sought to improve the nation's health by:

- Investigating the basic structure and functional mechanisms of the nervous system;
- Exploring the epidemiology and pathogenesis of neurologic and communicative disorders to facilitate prevention and early diagnosis;
- Developing improved pharmacologic, surgical and other therapeutic approaches through laboratory investigations and clinical trials;
- Disseminating new research findings to the health care community via timely publications and symposia; and
- Training highly qualified young physicians and scientists in research methodologies.

In each of these activities, the past year has been one of exceptional accomplishment.

Before reviewing this record of scientific achievement, it may be useful to focus attention on certain administrative issues now confronting the Program. The area of personnel management continues to pose the gravest concern. During the latter months of the past fiscal year, the ceiling for full-time permanent personnel was cut by another 13 positions to 282. Since no effective mechanisms exist to force the marginally productive tenured researcher from government service, the Program can do little more than attempt to achieve the lower ceiling through attrition. The resultant impact on scientific accomplishment is severe, since attrition acts preferentially to deprive the Program of its younger and usually most creative scientists who occupy short-term Associate or Fellow positions. To compensate for this as well as earlier personnel reductions, the Program has made strenuous efforts to recruit ceiling-free personnel, who are not NIH employees and thus do not occupy budgeted positions. In May 1977, a total of 511 individuals worked in the Program, an increment of 45 over the preceding year. A 45% increase in ceiling-free scientists largely accounted for this difference. Should the budgeted personnel ceiling remain static or be further reduced, additional attempts to use ceiling-free hiring mechanisms--particularly the Intergovernmental Personnel Act--will be initiated.

Several other administrative efforts, directed towards maintaining scientific excellence and productivity despite work force reductions, also deserve mention. In order to check the drift towards a largely tenured staff, severe restrictions have been placed on the conversion of younger researchers to regular staff positions. As a result, the proportion of tenured scientists within the Program has remained stable at about 47% of the total budgeted scientific staff. Concomitantly, attempts continued to provide more adequate support staffing for the scientific cadre. The past year witnessed further improvement in this regard, with a 5% increase in the number of budgeted and non-budgeted administrative/ technical personnel.

Another unremitting personnel problem concerns the recruitment of senior level scientists. The most conspicuous example of this difficulty has been the Program's inability during the past three years to fill the post of Chief of the Surgical Neurology Branch. All candidates identified by the Search Committee as highly qualified have refused offers of employment on the basis of insufficient compensation. University salaries received by these individuals exceeded by at least 40% the highest amount NIH could pay. A similar situation frustrated employment negotiations earlier this year with an accomplished mid-career biochemist-pharmacologist. The recent passing of Dr. M. G. Fuortes, distinguished Chief of the Laboratory of Neurophysiology, has created another major gap in the Program's scientific leadership which, under current circumstances, may be similarly difficult to fill.

Budgetary constraints also acted to limit the scope and efficiency of Intramural activities during FY 77. The Program's allotment for direct operations from October 1, 1976 to September 30, 1977 was $5.7 million, an increase of $.6 million over the preceding year. Although this allocation met the basic, recurring equipment and materiel needs of Intramural scientists, it failed to allow the Program to fully keep pace with major advances in neuroscience instrumentation and technology or to exploit many newly emerging opportunities in neuroscience research. The FY 77 Intramural budget also included a research contract allocation of $2.9 million. This allotment, up less than 1% from the previous year, afforded no growth in this important mechanism for supporting and extending the Program's in-house research activities.

In addition to personnel and budgetary constraints, limitations on laboratory and office space assigned to the Intramural Program also continued to hinder investigative activities. Although on-campus space occupied by the Program during FY 77 remained constant at approximately 90,000 square feet, the availability of two new off-campus facilities may be expected to help ease this situation. Renovations of Building 376 at Ft. Detrick, Maryland, are nearly complete. It is anticipated that portions of the Laboratory of Central Nervous System Studies will be transferred to this facility in early FY 78. In addition, the Institute's off-campus space assignment in the Park 5 Building, Rockville, Maryland, will exceed by approximately 3,000 square feet that lost by phase out of laboratories in the Auburn Building. Minor renovations of Park 5 are now virtually complete, and one Intramural component, the Laboratory of Perinatal Physiology, has already been transferred to this building. At least two other components--the Molecular Virology Section of the Laboratory of Molecular Biology and the Myelin and Brain Development Section of the Developmental and Metabolic Neurology Branch--are scheduled to move to Park 5 during FY 78. Collectively, the Ft. Detrick and Park 5 facilities will provide the Intramural Program with a net increment of nearly 40,000 square feet of critically needed space.

During FY 77, restricted availability of another vital resource--hospital beds in the NIH Clinical Center--served as the greatest hindrance to clinical research efforts. The Program was directed to reduce its use of assigned hospital beds to approximate the Clinical Center's 70% operational level and thus alleviate chronic nursing overload. Despite Intramural's record of efficient bed use (monthly occupancy approaching 100%), the number of beds available to Program clinicians thus fell from 52 to as few as 40. To counteract this situation, the Program attempted to rely more heavily on a Special Ambulatory Care Program, which allows research patients to be housed in commercial, off-campus facilities. The Program also sought to ensure maximum use of nursing staff through the appointment of a nurse supervisor to monitor personnel and bed assignments on its wards. At best, these steps provided only partial relief, and during the year many research protocols had to be deferred or discontinued. Hopefully, full implementation of a Clinical Center bed-banking plan will ensure a more equitable distribution of Clinical Center resources.

Despite the foregoing limitations, 152 Research Projects remained active; 42 new projects were initiated, 28 completed, and 15 terminated. Several of these studies involved collaborative efforts with other federal agencies and non-governmental institutions. Based on the judgment of scientists reporting on individual research projects, 57% of Intramural funds supported fundamental investigations, while 43% was directed towards clinically applied studies. These figures indicate that, in NINCDS, the proportion of Intramural support devoted to basic research exceeds that of most other NIH Institutes. Neurophysiology, neurochemistry, and bacteriology/virology/immunology received the largest budgetary allocations, while applied studies of demyelinating, infectious, and congenital/perinatal disorders were accorded the largest support in the clinical neurosciences. On the basis of research bed utilization in the NIH Clinical Center, studies of metabolic/developmental, neuromuscular, convulsive, and extrapyramidal disorders--as well as brain tumors--were most active.

Based on the record of scientific publications, Intramural scientists enjoyed another very productive year. During 1976, 281 research reports appeared in 137 different scientific journals and books. The greatest number of publications were in the areas of neurophysiology, neurochemistry, bacteriology/virology/immunology, and demyelinating disorders. Those journals publishing the bulk of articles authored by Intramural scientists were, in descending order, Brain Research, Neurology, Archives of Neurology, Advances in Neurology, Experimental Neurology, Science, The Proceedings of the National Academy of Sciences, USA, and the Journal of Neurosurgery.

Evidence of the high esteem accorded the Program's scientific performance derived from several sources. During the year, one Intramural scientist received the Nobel Prize for his revolutionary discoveries concerning the relation of slow virus infections to central nervous system disease. Others accorded public recognition for outstanding scientific accomplishments included seven Intramural staff members given the DHEW Distinguished Service Award or the PHS Superior Service Award. Rigorous triennial evaluations of Intramural Laboratories by the Institute's Board of Scientific Counselors, augmented by Ad Hoc specialists, also yielded highly laudatory comments, as well as average priority scores superior to those currently required by the Institute for funding of extramural research grants.

Details of the outstanding contributions made by Intramural scientists to new knowledge in the neurosciences appear in the Laboratory Chiefs' summaries and in the individual Project Reports. Nevertheless, a few of these accomplishments deserve special mention here.

Research in the Laboratory of Molecular Biology has helped to explain the mechanisms of virological autointerference. When viruses are reproduced, some defective particles develop that contain only a portion of the viral chromosomes, but all of the viral proteins. When both the complete virus and the defective particles simultaneously invade a cell, the defective particle interferes with replication of the infectious virus. Research has shown that the autointerference phenomenon is caused by competition between the interfering particle and complete virus for the enzyme RNA replicase and that the genetic portion of the particle is unimportant to autointerference. The terminal nucleotide sequences of the interfering particle are the same as those of the infective particle and thus competition likely occurs at binding sites on the enzyme. Several defective interfering particles characterized by the research are of great practical interest. Since they have the same protein coats as normal virus, interfering particles stimulate host defense mechanisms in the same way as infective particles and thus have potential use as vaccines to stimulate antibody production. Defective virus particles also have the ability to induce production of the antiviral protein, interferon. A particular defective particle discovered and characterized by researchers in the Molecular Biology Laboratory has been found to be one of the most potent inducers of interferon known; hence, application of this observation would allow more efficient production of interferon than is now possible. This finding is of such practical potential that a patent application has been made to protect the right of public use.

In the Laboratory of Central Nervous System Studies, researchers have established the existence of a class of virus-like infective agents that can cause chronic, progressive, noninflammatory diseases of the human nervous system. These diseases may be sporatic (e.g., Creutzfeldt-Jacob), epidemic (e.g., kuru), or familial (e.g., some forms of Alzheimer's disease). The unusual resistance of these agents to ultraviolet and ionizing radiation, to formaldehyde, or to heat set them apart from all other known microorganisms. Also unique is their ability to produce fatal CNS disease without eliciting inflammatory responses; the failure of the course of disease or incubation period to be influenced by immunosuppression; and the lack of any humoral or delayed hypersensitivity reactions during the diseases--a series of atypical biological properties. On the other hand, such properties as adaptation to new hosts, dependence on the genetic breed of the host, presence of strains of differing virulence in wild stock viruses selected by limiting dilution, and interference of fast growing by slow growing strains all point to a complex host-virus genetic interaction characteristic of more classical viruses. Attempts to delineate the chemical nature of the replicating agents--especially to determine whether they are replicated from introduced genetic information or by the induction, derepression or activation of pre-existing genetic information in the host--have been the major thrusts of current investigation.

Using the relatively new but powerful technique of freeze-fracturing, studies in the Laboratory of Neuropathology and Neuroanatomical Sciences have shown that normal structure of neural synapses during a variety of functional states can be defined, as can the different chemical types of synapses. These discoveries will assist significantly in determining the chemical organization of synapses in the central nervous system, and should make it possible to understand at a cellular level the action of drugs on the brain. Other researchers in this Laboratory are attempting to develop a diagnostic test for multiple sclerosis based on their finding that myelinotoxicity is present in the CSF of a majority of MS patients studied. Although further definition of specificity is needed, these developments offer hope for a definitive diagnostic test for multiple sclerosis in the future.

The Laboratory of Neural Control has demonstrated that cutaneous neural input pathways can preferentially activate high-threshold fast twitch motor units. The existence of such differential control systems is of great importance in the understanding of motor unit recruitments and motor control, a subject of clinical importance.

A cochlear carbonic anhydrase has been discovered, isolated and characterized by the Laboratory of Neuro-otolaryngology. Glutamic and aspartic acid have been identified as possible transmitters of the auditory nerve ending, while it appears as if other neural and acidic amino acids do not serve as neurotransmitters in the ear. Anatomical studies have shown that small cells previously thought to be interneurons project instead into the inferior colliculus. Although the functional significance of these new findings remains unknown, they portend a greater understanding of auditory function and dysfunction.

Research conducted in the Biophysics Laboratory has produced much new information on the neural basis of learning in lower life forms having simple nervous systems. For the first time, it has been possible to identify both anatomically and functionally the neural elements involved in a learning event. Use of the simple nerve system has also permitted identification of critical developmental stages for neural networks. The Biophysics Laboratory has been first to mariculture suitable lower forms (hermissenda crassicornis). This not only ensures a continuous supply of animals with a well defined simple nervous system, but also permits examination of neural aspects of learning during various stages of neural development.

The Neuroimmunology Branch in collaborative studies with the Laboratory of Molecular Biology has continued to examine virus-host interactions in various models of virus-induced CNS disease. After inoculation of mice with vesicular stomatitis virus, acute encephalitis and death occurred within five days. In contrast, animals receiving defective interfering particles together with infectious virus, developed chronic disease especially involving the spinal cord. It is not yet known whether the chronic disease results from modification of the immune response, or changes in the biology of the virus, or both. The Branch has also been evaluating immune functions in patients with clinically well-defined multiple sclerosis. Employing a newly developed assay, these researchers have found an antigen on the surface of B cells in approximately 70% of MS patients but in only 30% of control individuals. In addition, solid-phase radioimmunoassay has been developed for measuring anti-viral antibodies in CSF, with preliminary findings suggesting an increase in CSF antibodies against measles in most patients with MS.

In a collaborative perinatal research study conducted by the Infectious Diseases Branch, patients who had symptomatic, treated urinary tract infections during pregnancy were found to have increased rates of prematurity, abortions, still births and neonatal deaths. Since infections of this type occur in over 3% of all pregnancies, these observations are of considerable practical importance. During the past year the Branch has also perfected a new serologic assay for rubella antibody using the enzyme-linked immunosorbent assay. The test is highly specific and rapid, and should be most useful for detecting low levels of antibody in sera. Investigations carried out by the Branch have yielded important negative information relevant to the pathogenesis of multiple sclerosis. The report of a multiple sclerosis agent detected in PAM cells by non-NIH investigators was not confirmed. Similarly, the published observation that a multiple-sclerosis associated agent can be detected by mouse inoculations and polymorphonuclear white cell depression could not be duplicated. Moreover, although investigators elsewhere have reported measles fluorescence in the jejunum of multiple sclerosis patients, only nonspecific reactions could be found by workers in the Infectious Diseases Branch.

In the Clinical Neurosciences Branch a detailed study of chronic cerebellar stimulation in patients with intractable epilepsy failed to show any significant difference in seizure frequency between periods of stimulation and nonstimulation. These objective findings were in marked contrast to the highly favorable reactions of patients and their families to this procedure. An extensive study of seizures originating from the temporal lobe has yielded evidence that violent and automatic behavior is less characteristic of these discharges than previously thought. Basic investigations conducted by the Branch have involved an experimental model of acute, cortical epilepsy in cats, designed to determine whether amino acid antagonism or facilitation contributes to the epileptogenic action of penicillin. Since penicillin iontophoresis reversibly but nonspecifically antagonized the three amine inhibitory amino acids tested (glycine, alanine and GABA), it would appear that the primary mechanism for this type of epileptogenesis probably does not depend upon an alteration of specific amino acid responses.

The work of the Developmental and Metabolic Neurology Branch continues to be directed towards the control and treatment of inheritable disorders of the nervous system. New simplified reagents for the diagnosis of Neimann-Pick disease, Krabbe's disease and Gaucher's disease, which have been designed and synthesized by this Branch, are now in commercial production and widely available. These analogs of the accumulating lipids are also used for the detection of heterozygous carriers and for the prenatal diagnosis of these diseases. A high-yield, large-scale procedure is now operational for obtaining highly purified glucocerebrosidase for enzyme replacement trials in Gaucher's disease. And sphingomyelinase, the enzyme lacking in Niemann-Pick disease, has now been isolated in highly purified form from human placental tissue. Studies during the past year have shown that the mammalian blood brain barrier can be temporarily altered by intracarotid infusions of various hypertonic solutions to permit entrance of such enzymes into brain. Since previous enzyme replacement trials have been thwarted by the inability of exogenous enzymes to gain access to damaged central nerve cells, this newly developed procedure offers the first hope for development of specific therapeutic measures for the various enzyme deficiency diseases causing neurological dysfunction. Other research conducted by the Branch has dealt with the possible role of gangliosides as biotransducers of environmental signals that control cellular metabolism. For example, gangliosides have now been shown to be important components in cell receptors for thyrotropin, chorionic gonadotropin, and luteinizing hormone, and insight has been obtained into the interaction of these hormones with their target organs.

The Medical Neurology Branch has completed extensive correlative studies of the histochemistry and single-fiber-electromyogram in patients with various neuromuscular disorders. Ordinary neurogenous denervations were found to have significant densification of motor units electrically and fiber-subtype grouping histochemically. On the other hand, chronic myopathies had mild densification but no definite subtype grouping, thus indicating a greater sensitivity of single-fiber EMG recordings. Based upon their demonstration of extrajunctional acetylcholine receptors on muscle, the Branch has now formulated a law of "mal-innervation" according to which extrajunctional receptors may arise from neurogenous or myogenous de-innervation or dys-innervation, or from primary non-innervation.

Analysis by the Surgical Neurology Branch of results in over 100 patients who have completed trials of adjunctive therapy on malignant gliomas suggests a direct correlation between the effects of chemotherapy and grade of tumor. Not only does adjunctive chemotherapy appear less effective against the more benign grades of glioma, but it also now appears that such therapy may actually have a deleterious effect on all grades of glioma other than the most malignant. Research suggests that the reason patients with less malignant tumors do not benefit from additional chemotherapy is that such chemotherapy acts to depress cellular immune mechanisms and thus reduce long-term survival. Because such mechanisms are absent in the most malignant gliomas, chemotherapy cannot have any additional deleterious effect on the anergic immune system. In the field of computerized tomography, the Branch has demonstrated that protons can be employed to detect differences in tissue density at the 0.1% level--5 times more sensitive than that achieved by presently available commercial scanners.

The Experimental Therapeutics Branch has developed a novel method for studying neurotransmitter metabolism in the central nervous system of man using a non-radioactive isotope of oxygen. Other clinical studies have generated the first report of potentially serious hepatoxicity arising from administration of an ergot derivative, lergotrile. Without this observation, lergotrile would almost certainly have caused liver damage in a significant number of neurological and endocrinological patients; it is now most unlikely that lergotrile will ever be marketed. Recent clinical investigations using potent receptor agonists now indicate that prolactin release from the human anterior pituitary is under the influence of both GABA-containing and acetylcholine-containing neural pathways.

The Laboratory of Experimental Neurology has continued to study epilepsy in monkeys. By means of the radioactive antipyrine technique, increased blood flow has been demonstrated initially in the ipsilateral sensori-motor cerebral cortex and putamen, later extending further into subcortical structures. Regional changes in the concentration of neurotransmitters have also been investigated in this primate model of epilepsy. A moderate bilateral increase in dopamine has been found during the fit, together with an eightfold increase in gamma aminobutyric acid.

Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

July 1, 1976 - September 30, 1977

Theodore R. Colburn, Ph.D., Chief

The Section on Technical Development is a group of engineers, computer specialists, and technicians which provides technical services to the Intramural Research Programs of NIMH and NINCDS. A veterinarian and a group of laboratory animal technicians and animal caretakers provide animal medicine and care services to the Intramural Research Program of NIMH. The major functions of the Section are:

(1) Instrumentation research and development. Design and development of instruments and instrumentation systems which represent advances in the state-of-the-art. Most of the research within the Section falls in this category, and is generally done in collaboration with investigators in the laboratories of NIMH and NINCDS.

(2) Production of custom instrumentation. Design and fabrication of electronic, mechanical, and optical equipment to suit the particular needs of the requesting investigator. These instruments, while often quite complex, utilize rather than advance the current state-of-the-art in design techniques and components.

(3) Computer services. The Section assists the investigators in data collection, reduction, and analysis, by supporting two laboratory digital computers for general use, including real-time on-line applications, and by providing programming service and technical consultation.

(4) Laboratory animal medicine and care. The Section provides veterinary medical care and a centralized program of animal caretaking functions. A health care program for employees who work with animals is being developed.

Additional services provided by the Section include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether the Section will take on the project. In some cases it is more appropriate that the work be done elsewhere, due to the nature of the project or to an excessive backlog of work.

When the Section Chief agrees to take on a project, the investigator is required to submit a memo, initialed by his Lab Chief, which formally requests the services of the Section and provides complete specifications of the functions which are to be performed by the required instrumentation. Section personnel will respond in writing as to how the instrument is to be built, how much it will cost, and approximately when it will be ready. The Section does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Section's CAN.

Two essential changes have been made this year in the operation of the Section. Short-term mechanical jobs (four hours or less), which have an emergency nature such that an on-going research project is being delayed, are now being handled on a first-come, first-served basis by a technician assigned to that duty for a two week period. This service has been instituted so that an investigator need not wait two or three weeks (our typical backlog) to have a small job done if his research will be delayed. However, we wish to stress that this service is provided only for urgent projects, and must not be abused by persons who do not properly anticipate their instrumentation needs, or simply wish to circumvent the system.

The second change involves the auxiliary machine shop. Although the Section has traditionally provided a facility for investigators to do their own mechanical work in an unsupervised situation, this policy was reconsidered in light of the frequent abuse of the machinery (generally due to inexperience and haste) and disregard for minimal safety and cleanup standards. We now station the technician responsible for small jobs in the auxiliary machine shop; in general, he will do the work formerly performed by the investigator. In certain cases where the investigator insists on doing the work himself, and the technician feels that he is competent to do so, he will be allowed to use the machines under the supervision of the technician.

INSTRUMENTATION

The following are selected instrumentation projects undertaken during the past year. These are chosen from a total of 245 projects, and are representative of the types of electronic instruments and systems developed by the Section:

(1) Patient Activity Monitoring System. The Section has continued to develop a system for monitoring the activity patterns of ambulatory subjects. The system is built around the basic activity monitor, which was redesigned

this year. As the device is now configured, a piezoelectric accelerometer generates a voltage when the limb to which the monitor is attached moves. If the voltage exceeds a preset threshold, an event is recorded. A standard timing period (generally 15 min.) is divided into 4096 equal intervals of 0.22 seconds, and only one event can be recorded in each interval. At the end of the timing period, the total number of events, divided by 16, is stored in solid state memory; the event counter is cleared, and a new timing period is initiated. The memory can store activity totals for 256 timing periods (64 hours), and the range of activity counts for each period is 4096 ÷ 16 = 256.

Stored activity data is extracted from the monitor by reading the device directly into a PDP-11/40 computer through interface electronics. The computers are located in Building 36, Building 10, and the William A. White Building at St. Elizabeth's. With the computer handling the read-out, permanent storage, numerical processing, and plotting of the activity data, the need for manual data handling has been essentially eliminated. This development has been a major factor in the success of the activity monitoring programs thus far.

Other new developments in the system include:

(a) Portable Readout and Storage Device. In order to facilitate use of the monitors with subjects in remote areas, where access to the computer for data readout is impractical, a device was built into a briefcase to provide interim data storage. The device, designed and built to be operated by the subject, reads the data from the activity monitor and stores it on an audio cassette tape in a recorder which is concealed from the subject. Each tape holds about one year of data. The briefcase is returned to the investigator, who then plays the audio tape directly into the computer.

(b) Data Transmission by Telephone. An equally promising but potentially cheaper method of transporting data from remote locations is now under development. The device will read data from the activity monitor and transmit it by frequency shift keying over telephone lines to the investigator here at NIH, where the data will be stored on audio cassette tape until it can be read into the computer. Thus, long term outpatient studies can be conducted in any area with telephone service.

The activity monitor system has been well received by the technical community; over 35 monitors are now being used by seven different research groups. Applications include research on manic-depressives, hyperactive children, anorexia nervosa patients, drug addicts, and restrained monkeys. Publicity gained from word-of-mouth and from our two publications has evoked numerous inquiries from investigators all over the country. We have filed a patent application, and several firms have expressed interest in licensing the device for manufacture.

(2) Measurement of Activity of Confined Animals. The Section has continued to innovate techniques of transducing and recording quantitative and qualitative aspects of confined or restrained animals. The following are examples.

(a) Rat Activity Monitoring Cage. An activity cage has been developed which electronically senses the location of an unrestrained rat within the cage, the amplitude and frequency of gross and fine movements, and the occurrence of rearing. The instrument produces two analog voltages which together contain this spatial and temporal activity information.

Four capacitors are created by mounting conductive plates on the outside of each wall of the cage. The electrically grounded conductive floor of the cage is the common second plate of all four capacitors, which form a differential capacitor pair for each axis, X and Y, of the floor of the cage. These capacitors serve in the timing circuits of four one-shot multivibrators. As the rat moves within the four overlapping capacitive fields, he changes the effective size of each capacitor and thus, the period of each one-shot. Additional circuitry detects the period changes and produces the two analog output voltages, which indicate the instantaneous position of the rat in the X and Y axes.

One system is being developed which will include fifty of the activity cages interfaced to a PDP-11/03 microcomputer for data acquisition. Programs in the 11-03 will analyze the output signals from the cages in real time for types of activity pertinent to a particular study. Summaries of that information will be recorded on a digital cartridge tape unit. The cartridges will be transported to a larger computer to be read for further analysis.

(b) Measuring and Recording Long Term Rat Activity. A microprocessor based system for continuous monitoring of activity from 16 rat exercise wheels was developed. One full rotation of a wheel sets an appropriate flag bit; every ten seconds the microprocessor scans all 16 flag bits and stores the data on a digital tape cassette. Data analysis of the activity patterns is done off-line on the Section's PDP-11/40 computer. Programs were written to present the data in the classic format currently used for similar data taken from mechanical event recorders.

(c) Activity of Monkeys in Restraining Chairs. A system is being developed for periodic measurement and recording of activity and temperature data from as many as 12 monkeys in restraining chairs, for purposes of observing physiological rhythms. A thermistor affixed to the cortex transduces temperature, and a specially designed activity monitor mounted to the skull measures head movement. The data from all animals is periodically acquired by a microprocessor and stored on digital cartridge tape, for later analysis on a digital computer.

(d) Rat Rotation Monitor. This device transduces and counts the clockwise and counterclockwise rotations of a rat for neuropharmacological purposes, such as evaluation of the efficacy of catecholamine lesions. Four channels are provided with a single, central timing control for simultaneous processing of four animals.

Each animal is harnessed by a combination Velcro waistband and wire neckloop via a stiff spring to a mechanical-electrical interface. This interface converts each revolution to three distinct electrical pulses by means of slotted, optical-limit switches. The interface is positioned at the top center of the animal's vertical cylinder plexiglas cage.

The device encodes the three pulses to recognize only full revolutions and stores these revolution counts in two counters, one each for clockwise and counterclockwise. Total counts are summed for user selected intervals of five or ten minutes. Each count is sampled at ten second intervals, converted to an analog signal and output to the user's recording device, typically a strip chart recorder. An alternate digital output mode is available: each count pulse is presented at the time it occurs. This mode is TTL compatible.

(3) Event-Centered Raster Generator. This instrument detects a random event, such as a voluntary animal response, and produces a dot-intensified raster display that is centered in time on that event. Equal time segments of pre-event and post-event neuronal responses may be observed for up to 64 sequential events. For each raster sweep, the neuronal responses are displayed within 256 time bins, with the bin time selectable from 1 to 99 msec. LED displays show the number of raster sweeps and the total number of both pre- and post-event responses.

A secondary event, either synchronized with or independent from the primary event, may also be detected and displayed on the raster. Thus, for stimulus-response experiments, the raster may be centered on either the stimulus or response and the time correlation of neuronal responses to both of these "events" may be recorded.

(4) Discriminator and Iontophoresis Systems. The amplitude/time window discriminator system designed in 1973 (usually including an amplifier/filter, a raster generator, and a rate meter), and the multichannel high-voltage iontophoresis system designed in 1974 continue to find wide use. This year five discriminator systems and two iontophoresis systems were completed. These units bring the total number in use in the Intramural Research Programs of NIMH and NINCDS to 19 discriminator systems and 8 iontophoresis systems.

(5) Microelectrode Preamplifier. The latest IC operational amplifiers have been used to develop a battery-powered preamplifier for unit recording with metal or glass microelectrodes. The device has high input impedance, low noise, and selectable gain and bandwidth.

(6) Respiration Monitor. A previously developed respiration monitor for monkeys was improved by the use of a smaller and more sensitive thermistor. The thermistor is mounted in a small plexiglas protector and is positioned near the animal's mouth or nostrils to sample the inspired/exhaled air. This position generally requires the animal's head to be immobilized. The output can be either to a meter for qualitative observation or directly to an EEG recorder. Amplification is adjustable so that satisfactory results have been achieved for infant monkeys during surgery or adults in experimental situations. Circuitry can be included to sound an alarm when breathing ceases for a preset time interval.

(7) Rat Sleep Scorer. A device is being developed to categorize the bifrontal electrocortical activity as low, medium or high, the frontal-occipital EEG as low or high and the EMG as low or high. It is hoped that logical combination of these states will enable the user to identify and record 10 second epochs of sleep as wakeful, REM, or NON-REM.

(8) Finger Tremor Transducer. A device to detect and quantify the finger tremor of human patients with Parkinson's disease is being developed. Measurement of differential capacitance changes modulated by thumb or index finger movement will accurately locate and track the finger position and movement. Outputs will be analog for input to a strip chart recorder.

(9) Optokinetic Nystagmus Stimulator. This device was constructed for use with adult monkeys in restraining chairs. The unit consists of a 90 cm. diameter drum, support framework, a DC motor to rotate the drum, and a control box. The support frame and drum rest atop the animal's chair for testing and are easily removed. The inside of the drum is unobstructed for easy attachment of visual stimuli. Interval illumination is provided.

Controls permit selection of rotational direction and choice of several discrete speeds between 5 and 40 RPM. The control box is self-contained, allowing remote use.

(10) EMG Biofeedback Device. A prototype EMG biofeedback device was designed and constructed to be used in muscle reeducation for post-stroke patients. Although the device can be used for any EMG biofeedback application, it was specifically intended for use in reeducation of leg muscles. The device can be used in several different ways. A microammeter is provided which can give immediate feedback for training purposes; the movement of the meter dial is proportional to the EMG activity. The device can also be used for long-term feedback where the total EMG activity is integrated for the desired period of time up to approximately eight hours. A small digital display gives the user a number which represents the total integrated EMG activity over the selected time period. In this mode, the patient would wear the device; therefore, the instrument had to be small, portable, battery powered and easy to use.

(11) AR-11 Interface. The PDP-11 computer is equipped with a real-time analog subsystem that is used by many investigators for input and output of analog data to their computers. The AR-11 includes A to D and D to A converters plus a programmable real-time clock. Five interface panels were designed and constructed to take full advantage of the AR-11 capabilities and in some cases modify the output signals for use with specific scope displays. The interface panel makes possible easy access to the 16 channels of A to D provided by the AR-11 and also allows the use of the digital control lines that are available for the display scopes. In addition, multi-use Schmitt-trigger circuitry is provided that makes use of one of the system's interrupt control lines.

(12) Electromagnetic Muscle Stimulator. A prototype device was developed to provide mechanical stimulation of individual muscles in an intact awake behaving primate. A 3 x 6 x 15 mm steel bar is implanted longitudinally in the flexor carpi ulnaris muscle of the forearm. The monkey sits in a primate chair with the forearm passing through a solenoidal coil of wire. A pulse of current passed through the coil creates an axial magnetic field which produces a force on the implant and results in a mechanical stretch of the muscle, followed by a tendon jerk and a transcortical response. Further development of the system is directed toward increasing the force and minimizing the required input power, and in using various time varying currents for different effects.

(13) Data Acquisition System for Behavioral Observation. A microprocessor based cassette data entry system has been developed for use in behavioral observation experiments. The system utilizes two custom keyboards which observers will use to record behavioral events. The keyboards

have illuminated displays which indicate current behavioral trends or patterns. A microprocessor sorts, time correlates and writes keyboard data onto a digital magnetic tape cassette. Following the observational period, the tape is removed to a computer facility for information retrieval, processing and analysis.

COMPUTERS

The laboratory computer system developed by the Section on Technical Development provides a versatile means of computer support to the individual laboratories. The computer, as used in the Intramural Program, is a laboratory instrument, providing a means of data acquisition, both off-line and on-line, process control and data reduction. The laboratory systems applications range from real-time systems interacting with the experiment to reading the data stored in the memories of the Patient Activity Monitor. The smallest system consistent with the need of the laboratory is used, and data is further processed on STD's support computers or the DCRT facilities.

The aim of the Section has been to provide routines specifically tailored for the needs of the intramural program, and to furnish systems assistance to the investigators writing their own programs. The Section provides individual training and assistance to users. This has proven to be a more efficient means of training than conducting formal classes.

The installation and implementation of the laboratory computers has been well within the predicted time frame, and the Section is now exploring means of improving the resources available to the investigator and to lessen the investment of time necessary for a scientist to efficiently utilize the computer as a laboratory instrument.

Programming is now done primarily in FORTRAN. FORTRAN is a relatively old compiler that was designed to process mathematical equations. Computer manufacturers have standardized on this language, but it is awkward and not efficient for laboratory use. The language PASCAL has been purchased and is being studied in detail. It should reduce the amount of programming time needed and produce more efficient computer code. The concept of Structured Programming is being examined and its application to on-line, real-time programming should provide a more efficient means of handling complex programs as well as reduce the number of errors and the time spent debugging experimental routines. Methods of handling time series data are being investigated. There have been many recent advances in digital filtering, Fourier transforms and noise analysis which have direct application to neurophysiological data processing.

The Section conducted systems studies for the application of small microcomputers for fixed purpose, laboratory instrumentation. Two such systems have been ordered for the Laboratory of Clinical Psychopharmacology. One will provide a continual monitor of physical activity and brain temperature of a number of monkeys over extended periods of time. The other application will monitor the activity of rats living in the cages designed by the Section.

Additional computer-related projects include:

(1) Automated Analysis of Autoradiographs. An operator interactive system for the densitometric analysis of autoradiographs is being implemented for the Laboratory of Cerebral Metabolism. Under control of a PDP-11/03 computer, the information present in pictorial form in the autoradiograph is converted to digital densitometric data by a scanning microdensitometer and displayed on a video monitor. Standard image enhancement techniques, such as contrast stretching, density slicing and pseudo-color, in conjunction with a joystick-controlled outlining frame, will allow image features to be easily detected and isolated. Once a feature of interest has been isolated, the system will be able to compute its average density and area. A hard copy of the enhanced image may be obtained either by taking photographs from the video monitor or by using the Versatec printer/plotter, which is part of STD's Building 36 support computer.

(2) Membrane Activity of Neurosecretory Cells. Cells have been isolated that exhibit bursting pacemaker activity; some of these cells are responsive to peptide neuro-hormones. Voltage clamp experiments designed to investigate the conductances which produce the electrical responses are in progress. The data, currently stored in analog form on tape and analyzed off-line, consists of slow conductance variations combined with extremely fast changes during the bursting activity elicited by changes in membrane voltage. Digital techniques are being developed to separate and analyze these components in order to study the underlying mechanisms of chloride conductances and membrane permeability. The eventual aim of these programs is an on-line, real-time system allowing the scientist to interact with the experiment.

(3) Single Unit Studies. Hardware and software support of Laboratory of Brain Evolution and Behavior studies of single units in primate brain has resulted in the completion of the CAUDAR (Computer Assisted Unit Data Acquisition and Reduction) system. Personnel of LBEB developed the FORTRAN software package and the real-time data acquisition portion was developed by STD. The system has run successfully since June 1976 with continuing developments and enhancements up to the present time. It has been submitted to and accepted by National Technical Information Service.

ENGINEERING, COMPUTER and FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories.

LABORATORY OR BRANCH	HOURS	PERCENT
Neurophysiology, NINCDS	4774	15.29
Special Mental Health Research, St. E's., NIMH	4059	13.00
Clinical Science, NIMH	3196	10.24
Neurophysiology, NIMH	3023	9.68
Brain Evolution & Behavior, NIMH	2432	7.79
Adult Psychiatry Branch, NIMH	1694	5.42
Behavioral Biology, NICHD	1346	4.31
Experimental Therapeutics, NINCDS	1342	4.30
Neuropsychology, NIMH	1094	3.50
Cerebral Metabolism, NIMH	1046	3.35
Neurobiology, NIMH	975	3.12
Psychology and Psychopathology, NIMH	959	3.07
Neurochemistry, NINCDS	776	2.48
Central Nervous System Studies, NINCDS	562	1.80
Developmental Psychology, NIMH	544	1.74
Molecular Biology, NINCDS	531	1.70
General and Comparative Biochemistry, NIMH	476	1.52
Biophysics, NINCDS	464	1.48
Neurochemistry, NIMH	392	1.25
Intramural Research Program, NIMH	374	1.19
Neuro-Otolaryngology, NINCDS	371	1.18
Experimental Neurology, NINCDS	254	0.81
Neuropathology and Neuroanatomical Sciences, NINCDS	222	0.71
Intramural Research Program, NINCDS	82	0.26
Perinatal Physiology, NINCDS	76	0.24
Infectious Diseases, NINCDS	73	0.23
Medical Neurology, NINCDS	66	0.21
NIMH (Total)	20,264	64.94
NINCDS (Total)	9,593	30.75
NICHD (Total)*	1,346	4.31
	31,203	100.00

* NICHD loans the Section one position, and is thus entitled to 1700 hours of service.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-NS-01526-10-ODIR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Epileptic Neurons and Their Recurrent Axon Collaterals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. L. Li	Associate Neurosurgeon	IRP, NINCDS
OTHER:	A. F. Bak	Electronics Engineer	LNP, NINCDS

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NINCDS

LAB/BRANCH
Office of the Clinical Director

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Single electrical stimulus of varying durations and intensities or repetitive stimuli at varying frequencies were applied to a single neuron or to a group of neurons in the cerebral cortex. The responses of the neuron or neurons were recorded. It was found that there are different mechanisms dependent on the interaction of the neurons in the cortex and subcortical nuclei, by which surface epileptiform discharges of different patterns are produced. Furthermore, the neuron, which appears to be an active participant in the generation of surface paroxysmal waves, may become inactive when a surface paroxysmal epileptiform wave appears.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-NS-01526-10-ODIR

PERIOD COVERED
July 1, 1976 to September 30, 1977

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PI:	C. L. Li	Associate Neurosurgeon	IRP, NINCDS
OTHER:	A. F. Bak	Electronics Engineer	LNP, NINCDS

COOPERATING UNITS (if any)

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LAB/BRANCH
Office of the Clinical Director

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Single electrical stimulus of varying durations and intensities or repetitive stimuli at varying frequencies were applied to a single neuron or to a group of neurons in the cerebral cortex. The responses of the neuron or neurons were recorded. It was found that there are different mechanisms dependent on the interaction of the neurons in the cortex and subcortical nuclei, by which surface epileptiform discharges of different patterns are produced. Furthermore, the neuron, which appears to be an active participant in the generation of surface paroxysmal waves, may become inactive when a surface paroxysmal epileptiform wave appears.

PHS-6040
(Rev. 10-76)

Project No. Z01-NS-01526-10-ODIR

Project Description:

Objective: To study the electrophysiological properties of the individual nerve cells in the cerebral cortex under different experimental conditions.

Methods Employed: Glass micropipette electrodes were inserted into the cells in the motor cortex of anesthetized cats. The electrical activity of the cells was recorded; and, thereafter, pulses of electrical current of increasing intensity and of different frequencies were applied through the intracellular recording electrode, and the responses of the neuron were recorded. The electrical resistance of the cell was derived from the current-voltage ratio and the time constant of the cell membrane and capacitance were calculated. This was carried out before and after intravenous administration of various pharmacological agents or topical application of various convulsive agents. In some experiments, repetitive electrical stimulation was also applied to the cortical surface through a gross electrode adjacent to the recording micropipette electrode.

Major Findings: Some of the electrical properties of cortical neurons have been reported previously. The preliminary observations suggest that they are different from neurons under the effect of strychnine. For instance, the resistivity of normal cortical neurons measures (9.17 ± 2.68) x 10^6 ohms while that of the epileptic neuron was found to be in the order of 5 to 6 x 10^6 ohms. Further, epileptiform activity of the cortex requires "synchronous" presynaptic bombardments and there is good evidence of negative feedback through the axon collaterals to the epileptic neurons. Finally, the hypothesis or theory of Burns (1962) was tested. According to Burns' theory, repetitive discharges of a single cortical neuron would produce after-discharges which spread in all directions; thereby precipitating local epileptiform activity of the cortex. The present study proves that this theory is not valid, apparently due to the powerful negative feedback system among the cortical neurons. Reports of these findings are in process.

Significance to Biomedical Research and the Program of the Institute: The electrical properties of the cell determine the cell's "natural" behavior as well as its response to the environment. If the electrical properties of the epileptic neurons are different from that of normal cortical neurons, it seems likely that any effective pharmacological treatment will restore the normal electrical properties. Similarly, the electrical measurements may serve as a criterion of the effectiveness of the pharmacological agent.

Proposed Course of Project: The study will be extended to further investigate the mechanisms of epileptic discharges of cortical neurons and the mechanisms of the effectiveness of various pharmacological agents in the treatment of epilepsy.

Publications: Li, C.L., Okujava, V.M. and Bak, A.F.: Responses of Cerebro-Cortical Neurons to Electrical Stimulation with Particular Reference to Epileptiform Discharges. Exp.Neurol. 55: 173-186, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-NS-02010-05 ODIR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neurophysiological Mechanisms of Pain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	C. L. Li	Associate Neurosurgeon	IRP, NINCDS
OTHER:	G. Andrews	Visiting Fellow	IRP, NINCDS
	G. Mathews	Neurosurgeon	V.A. Hospital, Washington, D.C.

COOPERATING UNITS (if any)
Veterans Administration Hospital, Washington, D.C. and Laboratory of Neurophysiology, NINCDS

LAB/BRANCH
Office of the Clinical Director

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To study the fiber components which mediate pain sensations in the peripheral nerves, electrical stimulation and recording of the saphenous, splanchnic, cervical sympathetic and vagal nerve of the cat were carried out under various experimental conditions. Special emphasis was made in reference to the response thresholds, conduction velocities and magnitudes of the various components in the response to change of temperature, dosages of local analgesics and of anti-inflammatory agents.

PHS-6040
(Rev. 10-76)

Project No. Z01-NS-02010-05 ODIR

Project Description:

Objectives: To study the physiological mechanisms of pain and changes in pain responses to other sensory inputs and to pharmacological agents.

Methods Employed: The saphenous, splanchnic, cervical sympathetic or cervical vagal nerve was placed in a specially constructed stimulating-recording assembly. The nerve was stimulated through a function generator with negative or positive Haversine-wave of varying intensities and durations. After a series of control responses of the nerve were recorded, the nerve was exposed to different temperatures, to saline solutions of various tonicities, to xylocaine and to methylprednisolone acetate of various concentrations. Under these circumstances, the response thresholds, conduction velocities and amplitudes of the fiber components were again recorded until these components were completely abolished in the response. Thereafter, the nerve was replaced in normal physiological conditions and the recovery of the fiber components in the response was observed. In a second phase of the experiment, the various fiber components in the responses to stimulation were recorded at different levels of the central nervous system with gross and intracellular electrodes. Interaction of these responses with responses initiated from other nerves or from other cortical and subcortical neurons was studied. These responses were also studied under the influence of various anesthetics and Beta-Endorphin and other opiate-like substances.

Major Findings: Under similar experimental conditions, the amplitude and duration of a given component in the response of saphenous, splanchnic, cervical sympathetic and vagal nerves were different, but the response thresholds and conduction velocities of the recorded component were almost the same. It was also found that there was a large A-delta component in the sympathetic and splanchnic nerves; and a large C-fiber component in the vagus. Furthermore, there is a power function relationship between the conduction velocity and temperature; and within a range of 40 to 20° C, change in conduction velocity for each degree centigrade of the A-beta fibers was about 6.4 meters per second; of the A-delta, 0.85 meters per second; and of the C fibers, 0.08 meters per second. The A-delta fibers, as compared to the A and C fibers, were found to be most sensitive to temperature change, to analgesics and to anti-inflammatory agents.

Significance to Biomedical Research and the Program of the Institute: The present investigation may contribute to our understanding of the mechanisms and treatment of pain.

Proposed Course of Project: The present study is to be continued with particular emphasis on the cell activity and the electrical properties of the cell membranes in the central nervous system in response to pain and analgesic agents.

Publications:

Li, C.L., Mathews, G. and Bak, A.: The Action Potential of Somatic and Autonomic Nerves. Exp. Neurol. 56:527-537, 1977.

Li, C.L., Mathews, G. and Bak, A.: Effect of Temperature on the Response of Cervical Vagal Nerve. Exp. Neurol. 709-718, 1977.

Li, C.L.: Brève esquisse de l´histoire de la médecine chinoise avec allusion particulière à l´acupuncture. Le Mensuel du Médecin Acupuncteur. 31:3-9, 1976.

Mathews, G. and Li, C.L.: Common Errors in Pain Management. In Wittkower, E.E. (Ed.): A Psychosomatic Approach to Medical Practice. New York, Harper & Row, 1977, pp. 49-56.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-NS-01527-10-ODIR

PERIOD COVERED

TITLE OF PROJECT (80 characters or less)

Physiological Mechanism of Motor Function in the Cat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been temporarily suspended.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 00201 - 22 ODIR

PERIOD COVERED

July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Guam Research Center Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. N. Chase,	Director	IRP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work on this project has been transferred to Laboratory of Central Nervous System Studies (See Z01 NS 01282-13 CNSS).

PHS-6040
(Rev. 10-76)

Annual Report
July 1, 1976 through September 30, 1977
Medical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

W. King Engel, M.D.
Chief, Medical Neurology Branch

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The current techniques consist of: histochemistry, tissue culture, electronmicroscopy, immunology, autoradiography, biochemistry, and clinical neurophysiology. In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic, electrical, immunologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct and often new sub-forms; (c) specific or symptomatic treatment; and (d) induced animal models closely related to the human pathophysiologic states. Our main emphasis is on the neuromuscular diseases -- they are considered to be affecting more than 1,000,000 persons in the country.

For the clinical investigations, 394 patients were admitted for a total of about 7,325 patient days, and there were 1,012 outpatient visits. About 470 patient muscle biopsies were processed histochemically and reported out -- about 100 of those were from outside hospitals. Many other outside biopsies are submitted for formal opinion. Neurologic consultations were provided on 485 patients of other departments in the Clinical Center, with performance of indicated muscle and nerve biopsies, and electromyograms and nerve-conduction studies (>100 consult electrophysiologic studies). In the past year, 26 papers were published, 31 are in press, and there were more than 65 presentations to meetings.

The one-year approved residency training program in neurology was continued. Approximately 14 neurologists and other physicians and 9 technicians came this past year to learn clinical research techniques in neurology, especially in neuromuscular diseases and the application of enzyme histochemistry and special clinical electrophysiology thereto. A few residents from various other hospitals rotate through our service. The fifth annual 3-day course on neuromuscular diseases in Houston, in collaboration with the Veterans Administration and Baylor, was organized and most of the lectures given by our present and former staff. Five years ago we initiated (with IO-OD) the NINCDS videotape series of teaching lectures by putting the speakers of the first Houston symposium on tape -- there are now 55 tapes in our neuromuscular-disease series, seen by over 102,000 persons.

Many of our former trainees are full professors, associate professors, and assistant professors in academic departments, and many are directors of Muscular Dystrophy Clinics and Myasthenia Gravis Clinics; many are Medical Advisory Board members of the National Muscular Dystrophy, Myasthenia Gravis, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis associations.

A collaborative program on neuromuscular diseases with INSERM, Paris, through Hopital Salpetriere and Hopital des Enfants Malades is in progress.

Amyotrophic Lateral Sclerosis (ALS): Previously we have reported a neurologic abnormality with certain features like ALS in many cases of primary and secondary hyperparathyroidism; we have also studied a few cases with both frank hyperparathyroidism and frank ALS. To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have studied various parameters of calcium homeostasis. Reduction in whole body retention of radioactive calcium (>80 retention tests) was seen in patients with neuromuscular disease compared to normal controls. Reduction in retention in ALS patients was more pronounced than in myopathy and neuropathy patients. That reduction was due in part to inactivity because paraplegia regardless of etiology gave reduction in whole-body retention. ALS patients who were not paraplegic still had significantly reduced whole-body retention. That decrease was not due to decreased 25-hydroxy vitamin D levels, nor to resistance to dihydrotachysterol because administration of the latter corrected the decreased retention. Serum parathyroid hormone concentrations, measured, were higher than normal in 40-50% of all patients with neuromuscular disease and similar in all subgroups: ALS, myopathy, neuropathy. Response to calcium infusion will now be evaluated to determine if there is an abnormal response to imposed serum calcium fluctuations, because we have found that absorption of ^{45}Ca from gut was enhanced in ALS patients compared to disease-controls, yet serum calcium was normal and not different from disease-controls.

Folate (oxidized and reduced forms) and B_{12} in CSF and serum in sporadic and familial ALS was measured and compared to values in neuropathy and myopathy (with Johns Hopkins and Bronx VAH) and no significant reductions found when age and sex-matched groups were compared. Thus the posterior column degeneration in familial forms (originally described from this Branch) is not directly due to local reduction of folate of B_{12} (however, interference with its action on cells could be occurring). Additional data suggests that probenecid does not cause rise in CSF folate in ALS patients.

In ALS patients, including those with a late-post-polio progressive muscular atrophy or myopathy syndrome, we continue to search for evidence of a viral cause in ALS sera, CSF and tissues, by profiling of viral anti-bodies (with Johns Hopkins and IDB), and various tissue-culture techniques (including activation and evaluation by morphologic, antigenic, and reverse-transcriptase assays). To date, no viral evidence has been found.

Study of 50 ALS patients, age and sex matched with neuropathy and myopathy disease-controls (with IDB and Johns Hopkins) show no difference in serum antibodies to polio types 1, 2, or 3 nor of CSF antibodies indicating no significant local CNS production of poliovirus antibody. One subgroup with rapidly progressive ALS showed CSF antibodies which cross-reacted with rubella antigen -- we will now do radioimmunoassay of antibody levels to rubella and arbovirus antigens.

CSF IgA was found increased in untreated ALS patients compared with no-neurologic-disease and neuropathy and myopathy disease-controls (with IB); serum IgA also was elevated in those patients compared to the above controls. We are now determining whether there is a specific component of local IgA production in CNS reflected in CSF. Serum and CSF dynamics of IgA metabolism will then be studied. In 12 ALS patients, oligoclonal bands were sought in the CSF, but none found (with IDB). HLA-typing has not revealed any predominant types in our ALS patients.

As an induced animal-model (with LNC and Johns Hopkins), Swiss mice acutely infected with poliomyelitis, which attacks anterior horn cells, are being studied to acertain whether the time-course of reduction of neurotransmitters, substrates high-energy intermediates, and cyclic nucleotides shows an early drop, e.g., of cGMP, in spinal cord antecedent to paralysis, attempting to determine the sequence of the virus-induced biochemical alterations. We will also compare controls ± cyclophosphamide to polioinfected ± cyclophosphamide animals to eliminate the role of inflammatory cells which might have elevated cGMP.

From our continuing experience with more than 550 patients with ALS or ALS-like syndromes we emphasize that (a) about 10% of ALS is chronic, surviving 5-15 years or more (and therefore giving false hope to experimental therapists if they are not aware of this subgroup), and (b) as many as 15% or so do not have ALS and some are treatable, identifiable by careful clinical and laboratory examinations -- e.g., a patient this year presenting as ALS had posterior lumbar diverticula with ankylosing spondylosis.

Two extensive chapters are about to be published, one on numerous aspects of the biology of the lower motor neurons as a basis for understanding and investigating diseases thereof (>1000 references), and the other on various aspects of the several motor neuron diseases (>300 references), as well as 3 chapters on other neuromusular diseases: central core disease, rod disease, and muscle fiber hypotrophies.

Our new concept, introduced two years ago, developed on the basis of our combined EMG and histochemistry studies, our open-biopsy-EMG technique, and on theoretical grounds, that an EMG pattern on voluntary effort of brief-duration small-amplitude overly-abundant motor-unit action potentials (BSAPs), sometimes polyphasic (BSAPPs) is just as possibly a reflection of neuropathic as of myopathic diseases (and we criticized the established but vacuous cliche' of "myopathic EMG" for that pattern) becoming more widely accepted.

Two years ago we introduced the technique of α-bungarotoxin αBT) staining to demonstrate neurogenously deinnervated muscle fibers by their diffuse plasmalemmal extrajunctional receptors. Then, last year, we evolved the new concept of "myogenous mal-innervation" (in press), based on our demonstration that: (a) diffuse extrajunctional ACh-receptors were present on "regenerating-degenerating" muscle fibers in myopathic biopsies of our patients, (b) human, rat, and chick embryo skeletal muscle grown in tissue culture without innervation (pre-innervated muscle) has diffuse extrajunctional ACh-receptors, and (c) in adult animals, segments of muscle fibers experimentally separated from their motor innervation point but remaining viable develop diffuse extrajunctional ACh-receptors. Thus we have formulated a "law of mal-innervation", since it becomes evident that the presence of diffuse extrajunctional AChR in muscle fiber plasmalemma is certainly a sign of mal-innervation of the muscle fiber but it is not pathogenesis-specific since it can be either a neurogenous or a myogenous de-innervation of dys-innervation, or a primary non-innervation. Experimental myogenous deinnervation, as we reported last year, results in diffuse extrajunctional AChR, with which we are now correlating the appearance of fibrillations. In the cultured non-innervated muscle fibers of chick, rat and human tissue, the αBT-demonstrated diffuse plasmalemmal acetylcholine receptors (AChRs) of single myoblasts through multinucleated well-differentiated contracting fibers did not appear electronmicroscopically to have "hot-spots", as reported by others from light-microscopic autoradiography, -- we suggested "hot-spots" may be artifacts based on subtle plasmalemmal folds.

Correlations of histochemistry and single-fiber-EMG (SFEMG) have been made in patients with various neuromuscular diseases. Ordinary neurogenous deinnervations have significant densification of motor-units (from collateral foreign reinnervation of orphaned muscle fibers) and fiber-subtype grouping histochemically. Chronic myopathies (Duchenne dystrophy, polymyositis/dermatomyositis, limb-girdle morphologically-non-specific myopathy syndrome) had mild densification by SFEMG but no definite subtype grouping histochemically, indicating a greater sensitivity of SFEMG. From study of 8 patients with muscle fiber-type predominance without major evidence of ordinary denervation (5 central core disease, 1 congenital rod disease, 1 type-I-fiber-hypotrophy-with-central-nuclei, 1 type-I-fiber-predominant unclassifiable neuromuscular disease) it became evident that, in a field of fiber-type predominance, subtype-grouping is requisite for a histological diagnosis of neurogenous deinnervation (although its lack does not, hypothetically exclude an abnormality of LMNs).

Biochemical aspects of neurogenously de-innervated muscle, such as increased guanylate cyclase activity, are in our myopathy project.

Animal muscle spindles exhibit variations in the histochemical profiles along the intrafusal muscle-fiber length -- e.g., with the myofibrillar ATPase reaction the nuclear-bag-2 fiber stains darkly in the spindle-capsular region but lightly in the spindle-polar region. The significance of this variability is not known -- it may relate to the complex sensory and polyneuronal motor innervation of the intrafusal fibers. A current study of human muscle is correlating spindle-fiber histochemistry and electronmicroscopy, using matched interrupted serial-sections along the whole fiber length of the same spindle for fresh-frozen histochemistry and EM. Experimental motor deinnervation and reinnervation of animal spindles did not result in evident histochemical changes in contrast to extrafusal fibers, suggesting possible specificity of motor-axonal reinnervation or a trophic role of sensory innervation.

In patients with scoliois, our continuing studies show a wide variety of neuromuscular diseases (by muscle biopsy histochemistry) associated with and probably causing scoliosis, most commonly some form of neurogenic muscular atrophy. Some of our scoliosis cases were previously considered "idiopathic", a group we are studying in more detail.

Polyneuropathy (Peripheral Neuropathy) (PN): The peripheral neuropathies comprise a group of disorders of various causes, more than half unknown. They always cause serious physical handicap sooner or later in the course of the disease, sometimes associated with intractable pain and ulceration and loss of feet and hands. Our studies seek to delineate the underlying causes and where possible develop a treatment. We also seek fuller understanding of the basic biology and pathologic responses of the lowor motor and sensory neurons and peripheral nerves.

In the majority of patients we see the PN is of undiscernable cause. Those which are non-familial we may treat with LT-HSDAD-prednisone, especially if less than 5-years duration postulating a dysimmune pathogenic mechanism. This year we have formally summarized our ongoing experience in 25 carefully-documented patients who have had good to outstanding success, most having been given-up on by others and some having come with diagnoses of non-treatable diseases (e.g., ALS or "Charcot-Marie-Tooth" disease). Long-term treatment is required -- too-rapid reduction of dosage too soon results in exacerbation. Excellent results have been sustained for as long as 12 years in an adult and 9 1/2 years in a child (who at age 21 is still regaining motor skills). Our correlative studies indicate that patients most likely to respond have the triad (i) being dysschwannian in type (slow nerve-concuction times, (ii) relapsing, (iii) with elevated CSF protein, but even some non-relapsing patients (i.e., 2 with progressive course > 1 yr) without slowed nerve conduction times and with normal CSF have responded to LT-HSDAD-Pred. Prednisone has a broader scope of responsitivity in this disease than previously believed. Virtually all our corticosteroid-responsive patients are cortiosteroid-dependent requiring 5-20 mg single-dose q.o.d. to prevent exacerbation.

We have studied the mechanisms of HSDAD-Pred anti-dysimmune effect on circulating blood lymphocytes (see Myasthenia Gravis project) and its effect on cerebrospinal-fluid (DSF) lymphocytes and immunoglobulins. We developed a method to identify T- and B-lymphocytes in CSF and found percentages normally are the same as in peripheral blood (72 and 16%). In 9 chronic idiopathic relapsing polyneuropathy patients, with 2-10X elevated CSF IgM and or G, studied longitudinally, HSDAD-Pred caused reduction in CSF T- and B- lymphocytes and IgM, IgG and IgA, and serum IgG but not IgM or IgA; thus the accompanying remarkable clinical improvement may have been by prednisone affecting lymphocyte numbers and Ig production (with 1B) directly within the CNS. Our current studies of the pathogenesis involve investigation of oligoclonal Ig bands in the CSF and serologic investigation of sera and CSF re. acute/chronic viral infections using antibodies to 17 agents.

An often undiagnosed cause of sensory-greater-than-motor neuropathy beginning in adulthood is "idiopathic" amyloidosis. We reported the combination of crystal violet stain with fresh-frozen sections of a muscle biopsy (proven by us by more than a decade of use) as the preferred method of diagnosing this disorder. We reported our most recent 10 cases of non-familial amyloid polyneuropathy, the onset we reported of which was in later adulthood (mean age 54); 8 were male. We emphasized that this disorder is associated with, and probably the result of, a plasma-cell dyscrasia, detectable in 8/10 of our patients as multiple myloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments (IgG-kappa >> IgG-lambda, IgM-lambda). We propose that the neuropathy is due to a systemic metabolic abnormality, possibly related to a circulating abnormal protein fragment (i.e., a "para-Sparafucile" phenomenon, v.s.), rather than to pressures from multifocal amyloid deposits of immunoglobulin fragments or ischemia. Our treating 6 amyloid patients with melphalan, an "anti-myeloma" agent, was not of obvious benefit. We have also reported a new application of ^{99m}Tc-diphosphonate clinical radioisotope scanning (with NM, CC), as a diagnostic technique for identifying amyloid in soft tissues -- highly suggestive of this diagnosis is (in the presence of a normal soft-tissue x-ray) a diffusely positive scan, presumably based on binding of the tracer to cations that are binding to anionic groups in amyloid fibrils, a mechanism supported by our published EM histochemical and autoradiographic demonstration of calcium localized to amyloid. It also diagnoses cardiac amyloidosis in patients with "idiopathic cardiomyopathy". When quantitated it can be used to follow effect of treatment.

We have now established combined studies of patient sural nerve biopsies in vitro -- including in vitro nerve conduction velocities of fast and slow fibers, teased fiber histochemistry, electronmicroscopy, and EM-histochemistry, allowing more precise and direct multidimensional analyses of the afflicted nerves in polyneuropathy patients. This has shown, for example, marked slowed conduction in small (C) fibers correlated with small-fiber pathology when clinical conduction times (which do not "see" C-fibers) were normal. We have also established tissue-culture growth of human schwann cells from pathologic human nerve biopsies -- this will provide opportunity to directly study the pathologic cell growing in vitro and dysschwannian neuropathies such as dysimmune ones (e.g. "Gullian-Barre"), in diabetes mellitus and in metachromatic leukodystrophy. We are attempting to develop immunochemical methods to selectively identify individual schwann cells in culture, and will attempt to clone and produce a cell line of some pathologic human schwann cells.

Anterograde axoplasmic transport of protein, glycoprotein and other substances from the neuron soma to the periphery is critical in maintaining integrity of the peripheral nerves. There is also a retrograde axonal transport, the normal role of which is uncertain but, we hypothesize, may serve for: (a) continuous monitoring by the soma of its distal self (e.g., regarding the necessity or not for axonal regeneration), (b) monitoring the external environment of its tip, and (c) acquisition of nutrients; it may also serve as the mechanism by which toxic substances are acquired (v.i.). We have published our autoradiographic studies (with Johns Hopkins) demonstrating in animals a rapid anterograde transportation to the axon synaptic terminal at the neuromuscular junction and accumulation there of large amounts of protein and glycoprotein synthesized in the lower motor neuron soma from precursors leucine and fucose injected into the ventral horn less than 24 hrs. earlier. This demonstrated that these rapidly transported axonal proteins and glycoproteins go rapidly to the geographic location where they can be used, hypothetically, for (a) remodeling of the axonal tips and (b) trophic influence on muscle -- both possibilities we are studying further. Combined electronmicroscopy and autoradiography demonstrated that ligature of the nerve interrupted both anterograde and retrograde rapid axonal transport, causing intra-axonal accumulation of masses of smooth vesicular membranes on both proximal and distal sides of the ligature and of substances labeled from precursors injected centrally or peripherally respectively. Those results suggested that fast anterograde and retrograde axonal transport are very similar processes carrying predominantly membranous organelles and constituting a system of bidirectional fast transport (which is interrupted focally by the nerve ligation). Further, pathologic axonal swellings (at the cut ends of axons) were interpreted as the consequence of the acute focal interruption of this rapid transport system. Thus studies of axonal transport provide a means for investigating the origin and fate of axonal organelles in pathologic process. Our autoradiographic studies have also shown that fast anterograde axonal transport provides a necessary contribution to motor nerve regeneration in experimentally sectioned nerves.

A new principle/model for inducing an experimental allergic neuropathy (EAN) in animals is being reported. It utilizes immunization with soluble nerve protein fraction (in contrast to lipid-associated protein of myelin used in previous EAN models). This represents a new potential model of some human dysimmune-dysneuronal peripheral neuropathies such as in some patients we have seen with prednisone-responsive neuropathies without demonstrable schwann-cell involvement. It also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and parainfectious encephalopathies. Since our EAN animals also have a component of blockade of neuromuscular transmission that is responsive to edrophonium, the model may have some relevance to myasthenia gravis or other disorders of the neuromuscular junction.

Central Nervous System Disorders: Of relevance to the group of spinocerebellar degenerations comprises diseases of various causes, a few known, most not, which always result in serious physical handicap sooner or later in the course of the disease, and sometimes mental deterioration. Our studies seek to delineate the underlying causes, where possible attempt to develop a treatment, and define basic cellular pathophysiologic mechanisms.

cAMP is thought by some to be a mediator of synaptic transmission of some systems in the cerebellum. Our newly developed histochemical technique for its synthesizing enzyme, adenylcyclase (AC), shows the greatest amount to be in cerebellar blood vessels, and of the neuroal-associated enzyme the greatest amount was in the basket-cell basket endings at the base of the Purkinje cell, not in the Purkinje-cell soma as previously supposed from tissue-slice biochemistry -- this necessitates modification of current hypotheses of Purkinje-cell function. It also shows significant AC in blood vessels, which would influence biochemical assays of homogenates of microdissected tissue samples.

In Cockayne's syndrome (cachetic dwarfism, various neurologic dysfunctions, cutaneous sunlight sensitivity, recessive inheritance), the syndrome of normal-pressure hydrocephalus was discovered in all 4 patients studied (with NCI).

Progressive spastic paraplegia is a progressively crippling disorder of children and adults. The causes are not known. In press is our identification of three unrelated patients with a syndrome of chronic adrenal insufficiency from infancy juvenile-onset of progressive spastic paraplegia and dysschwannian peripheral neuropathy, with normal intelligence (with NIAMDD). A single metabolic defect is postulated to underlie the abnormalities in the neural and adrenal tissues (? an adrenoleucodystrophy variant).

Opthalmo-neurology: We have shown that the various neuromuscular disorders affecting the eyes, assuming they are correctly reflected by their limb-muscle pathology, can be on a neuropathic or myopathic basis. They cause various degrees of handicaps. Our studies seek to delineate the underlying disorder, analyze the neuro-opthalmologic defect, and, if possible, seek methods of treatment. We also seek fuller understanding of the basic biology and pathologic responses of the eye neuromuscular apparatus. The commonest associated limb-muscle pathology of the progressive external opthalmoplegia syndrome (in our series of 46 patients, after myasthenia gravis and myotonic atrophy are excluded) is a syndrome characterized by "ragged-red" muscle fibers in limb muscles, whether or not the limbs themselves are weak. Those ragged-red fibers, containing several types of mitochondrial abnormalities, have been studied intensively (see our Myopathy project). Other limb muscle pathology associated with progressive external opthalmoplegia in our cases includes (i) a vacular myopathy-plus-neuropathy, (ii) type-I-fiber-hypotrophy-with-central-nuclei, (iii) only denervation, (iv) only morphologically nonspecific myopathy, and (v) type-II-fiber smallness. The histochemical fiber-typing of normal animal eye muscles and the different post-denervation response of extrajunctional receptor distribution of those fiber-types is in press (see Myopathies project). Abnormalities of eye movement in the spinocerebellar ataxias and in myasthenia gravis are discussed above. The ocular manifestation of acute pandyautonomia have been described (with NEI).

Some patients with spinocerebellar degeneration have slow eye movements. We reported (with NEI) that they made abnormally slow refixational eye movements by the saccadic system (i.e., they were slow saccades) rather than by the voluntary pursuit system. That led to the proposal of a new conceptual scheme of how both normal and defective saccadic eye movements might be generated. Thus, quantitative recording of eye movements in a group of related patients with familial late-onset cerebellar ataxia has revealed new information about non-visual control of eye position, since the striking abnormality was a defective smooth-pursuit and fixation system. The patients showed evidence of various non-visual mechanisms of maintaining eye position that have not been previously delineated. Rebound nystagmus was shown to occur in normal individuals if fixation is eliminated -- however it becomes clinically apparent in patients with spinocerebellar degeneration because of a coexisting defect of visually-mediated fixation mechanisms. Accordingly, rebound nystagmus can be interpreted as a manifestation of one of the brain's compensatory mechanisms for maintaining eye position when visual systems are ineffective. The findings supported the suggestion that the cerebellum specifically: (a) helps maintain eccentric gaze; (b) produces smooth-pursuit eye movement; (c) modulates the amplitude of saccadic eye movements.

EM-histochemistry for calcium (with NEI), now reported, has shown its discrete localization within the sacs of the outer segment of the rod cells of the retina, suggesting that the sacs may have a function of calcium storage-and-discharge with photoexcitation analogous to that of the sarcoplasmic reticulum of muscle. The finding has importance in the hypotheses of photo-electrical coupling mechanisms.

Progressive blindness was reported in two children with acute lymphocytic leukemia intensively treated with chemotherapy and radiation and considered a possible complication of that therapy (with NEI).

Other: We have formally called attention to the possible menstrual hemorrhagic effect of aspirin incorporated into virtually every over-the-counter anti-dysmenorrheic preparation, on the basis of aspirin's known ability to reduce-clotting/increase-bleeding.

Myopathies are non-neurogenic, primary or secondary diseases of muscle. Some, such as the dermatomyositis/polymyositis group, are often at least partially treatable but their cause and details of their probably "dysimmune" pathogenesis are not known; others are not treatable but their cause is known, e.g., genetic deficiencies of phosphorylase, phosphofructokinase, acid-maltase or carnitine-palmatyl-transferase; while still others, such as Duchenne muscular dystrophy and others bearing the name "dystrophy", are of unknown pathogenesis and are untreatable.

The polymyositis/dermatomyositis (PM/DM) disease-complex is an acquired disorder causing progressive deterioration of muscle in children and adults. The primary cause is not known but the pathogenic mechanism is considered "autoimmune" or "dysimmune". All patients were seriously incapacitated and many died before the introduction of anti-dysimmune therapy. Our studies involved improving diagnositc methods, seeking the cause, analyzing the pathogenic mechanisms, and improving methods of treatment.

The special variation of anti-dysimmune treatment we introduced to this disease 8 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) has continued to be, in our series (about 70 cases) and others, the single best available treatment for children and adults (without or with an associated cancer). It has the greatest benefit/side-effect ratio and is easiest to manage. However, because not all patients respond and because the side-effects of LT-HSDAD Pred can be significant, we are defining some of the parameters of their immunologic response to prednisone and seeking predictive indicators thereof.

By use of T- and B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have established that in PM/DM patients, while HSDAD-Pred is clinically cumulatively effective for months and longer its measurable effect on the peripheral circulating lymphocytes, using currently available techniques, lasts less than 24 hrs; and these effects are more prodound on the T-lymphocytes. We are now correlating these data with concurrently obtained blood levels of prednisone and prednisolone to determine any hypoabsorption or hypercatabolism of the drug (with P,CC). In patients, children and adults inadequately responding to HSDAD-Pred we have found previously on an occasional-case basis some remarkable therapeutic responses to added azathiaprine (3 mg/kg/d) -- to more clearly establish the efficacy of azathiaprine (plus an unchanged dose of prednisone), a double-blind trial is being conducted in otherwise refractory DM/PM patients, 9 to date. We are also measuring the patients' lymphocyte responses to azathiaprine.

Pulomonary interstitial fibrosis we have found to be common in our DM/PM patients -- detailed pulmonary functions are now being evaluated in all our DM/PM patients, 11 to date (with NHLI). Cardiac involvement (condution blocks, arrhythmias, systolic mitral prolapse) was demonstrated in the majority of 20 cases of DM/PM studied with non-invasive cardiologic techniques with NHLI, VA Hosp. DC, and Georgetown Univ.).

DM/PM is presumably a dysimmune response to an exogenous antigen, specific treatment of which could be curative (as opposed to the merely suppressive action of all current treatments). We are now seeking that antigen, and some preliminary clues have been found.

Massive subcutaneous calcification can be a crippling complication of childhood DM. We have found a new way to clinically detect it in its early stages, isotopically with ^{99m}Tc-diphosphonate body scanning (with NM,CC) (for new microscopic detection methods, v.i.). Our therapeutic trials with diphosphonate have not been beneficial (also not beneficial in a patient with myositis ossificans). However, in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathiaprine-HSDAD-Pred program. The reasons for the calcification are being sought by our histochemical, electronmiscroscopic, and autoradiographic studies (v.i.).

The exact mechanism of muscle damage in PM/DM is unknown. Previously we have reported immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, findings supporting our earlier hypothesis that an aspect of the muscle damage may be vascular. While disorders of both immunoglobulins (produced by B-lymphocytes) and direct immunocyte toxicity (T-lymphocytes) might be occurring in all cases of PM/DM, perhaps the former (deposited as intravascular immune complexes) are more muscle-damaging in the childhood form and latter are more pathogenic in the adult form. We are now attempting to correlate a possible vascular mechanism with our histochemical finding of preferential perifascicular involvement (occurring especially in childhood DM), as well as relating our findings in patients to our experimental animal model of muscle ischemia (v.i.). It is conceivable that the immunologic abnormalities of PM/DM are ultimately provoked by a viral infection; however, our attempts to see or "rescue" a virus from PM/DM patients' muscle directly after tissue culture of it, or after animal inoculation, thus far have been negative with ID-NINCDS and NCI); nor has reverse transcriptase been found so far.

A new and possible disease-characteristic histochemical finding in PM/DM patients of microscopic foci calcium accumulation in collagenous connective tissue of muscle and subcutaneous regions, demonstrated histochemically at both the light-microscopic (alizarin-red method) and EM (pyroantimonate method) levels is being published. Its pathogenesis is being sought, as is its correlation with our positive ^{99m}Tc-diphosphonate patient-scans for calcium, our autoradiographs of patient skin and muscle biopsies for calcium, and our previously described highly-disease-characteristic histochemical finding of alkaline-phosphatase staining in the intramuscular connective tissue (also being published).

Duchenne muscular dystrophy (DMD) is an hereditary progressive deterioration of muscle in children, usually causing wheelchair or bed confinement by age 12 and death by age 20 years. Cause and treatment are not known. Our studies involve improving diagnostic methods, seeking the basic cause, and trying experimental therapy when available. We also seek fuller understanding of the basic biology and pathologic responses of muscle. The other myopathies and the other neuromuscular diseases of unknown nature which we study are often devastating to children or adults, and in studying them our general objectives are the same.

Current competing hypotheses of the pathogenesis of DMD are: (a) primary or secondary defect of blood supply to muscle, (b) primary defect of energy source within the muscle fiber, and (c) primary muscle-fiber plasmalemmal defect. Although many others favor c, we favor a or b. Some of the findings in DMD muscle considered by others to be supportive of c we consider to be invalid results or not distinguishing between c and b. For example, the defect of erythrocyte morphology reported by others we have reported as not present in our controlled studies. A further aspect of erythrocyte normality is our finding that in 8 DMD patients, ^{51}chromium-labeled erythrocyte survival was normal (with H,CC). The defect of the muscle-fiber plasmalemma evident by peroxidase penetration reported by others we are reporting not to be disease-specific. The alteration of muscle homogenate adenylcyclase reported by others we have reservations about because (i) it was not exclusive to DMD, and (ii) its cellular site (presumed by them to be muscle-fiber plasmalemma) could not be determined from those biochemical studies. Our own newly developed histochemical methods for adenylcyclase (AC), utilizing AMP-PNP as the substrate and Ca^{++} (which doesn't inhibit AC like Pb^{++} does) as the capture agent, shows highest muscle AC in blood vessels and almost none detectable in normal muscle at shorter incubation times; but in regenerative fiber fairly high amounts of AC are evident diffusely throughout the T-tubule (± SR) network and plasmalemma. This technique we also developed at the EM-histochemical level and showed AC precisely localized to the plasmalemmal and T-tubule membranes. In DMD biopsies, many fibers, often appearing normal with other histochemical reactions, show subtle regenerative features with the AC histochemical techqnique.

In DMD the plasmalemma has long been known to be leaky, evidenced by elevated CPK and other "muscle enzymes" in the serum, but that certainly does not specify a primary plasmalemmal defect (v.i.). Human regenerative fibers have certain fetal isozymes -- e.g., LDH-1,2 has been known and we now confirm the recent finding of CPK-MB. We have introduced the use of elevations of CPK-MB isozyme in serum (and in muscle biopsies) for detecting DMD carriers. Our other new method of carrier detection is elevated serum hemopexin (v.i.). These together we use as fine-tuning for DMD-carrier detection, identifying carriers missed by all other carrier-detection methods. Our data are also being reported in the context that elevated plasma CPK-MB is not diagnostic of myocardial infarction as some cardiologists have reported -- we find it elevated in any kind of leaky skeletal-muscle disease (e.g., DMD and DM/PM). Our new test of ^{99m}Tc-disphosphonate forearm scanning 24 hrs after ischemic exercise (6 hrs after isotope injection) did not detect DMD carriers (or patients) like it did phosphorylase and phosphofructokinase deficiency patients, (with NM, CC).

Hemopexin, an inducible, liver-produced, heme-transport protein, was first discovered elevated in DMD patients and carriers by Askanas a few years ago. We have confirmed that, and supported the hypothesis that the elevation is due to subtle myoglobin leakage from minimally damaged muscle fibers by finding: (a) with a sensitive radioimmunoassay, myoglobin leakage into the serum of nearly all DMD patients and half of the carriers (with Columbia); (b) hemopexin elevation in monkeys with experimentally crushed muscle or injected with myoglobin, which persisted long after CPK levels returned to normal (in the crush studies). A large number of neuromuscular-disease patients were surveyed with the immunodiffusion assay for hemopexin quantitation. Only active myopathies, especially dermatomyositis/polymyositis and DMD patients and carriers, and myasthenia gravis patients, had hemopexin elevations (since myasthenia gravis patients have no evidence of muscle-cell leakage the possibility of their hemopexin elevation being a very special dysimmune phenomena is being investigated). We are beginning to study hemopexin molecular turnover in a variety of neuromuscular-disease patients.

Our new model involving the blockage of leg-muscle anaerobic glycolysis by intra-aortic iodoacetate, which results in the secondary loss of plasmalemmal membrane integrity of the damaged fibers (v.i.), establishes an experimental basis for at least the possibility of hypothesis b re. DMD pathogenesis (above), in harmony with our clinical demonstration that in muscle phosphorylase deficiency ischemic exercise precipitates acute breakdown of the plasmalemmal barrier, allowing marked creatine-phosphokinase (CPK) egress and calcium ingress (detected by scanning with ^{99m}Tc-diphosphonate).

Our ischemia hypothesis for DMD, which proposes a functional defect on the arterial side of the vascular tree, has been based on our studies of the histochemopathology of DMD muscle and of our experimental ischemic myopathy in animals. As yet, an ischemia mechanism, although possible in DMD patients, has not been demonstrated in them directly (that would be the next logical step). However, our study in press demonstrates that in known ischemic human muscle, caused by occlusive vascular disease, the histochemical pattern of the regions of lesser involvement of muscle (groups of necrotic or regenerating fibers in fields of normality) is indistinguishable from that of early DMD and of our experimental ischemic myopathy in animals, thus being at least harmonious with the ischemia hypothesis (with VAH, D.C.). To date, none of the studies attempting to detract from our ischemia hypothesis is conclusive, nor even incisive.

A new method to measure capillary blood flow, utilizing the Doppler effect, has been developed by others. When another machine becomes available we will measure muscle blood flow directly during our diagnostic open muscle biopsy, in DMD, DM/PM and other neuromuscular diseases in which ischemia mechanisms have been considered. Platelet uptake of serotonin, which 10 yrs ago we found impaired in DMD, is being re-measured with a new technique.

Although phosphodiesterase (PDE) inhibition is considered to reduce peripheral arteriolar resistance and increase peripheral blood-flow, a PDE-inhibitor has not benefited our DMD patients; nor has allopurinol.

Using the ischemia-myopathy model to study details of the mechanisms of muscle fiber damage, we have reported the development of a new, rapid and simple radioisotopic method, based on uptake of ^{99m}Tc-diphosphonate, of quantitating active skeletal muscle damage in experimental animals -- that uptake was directly correlated with other parameters of muscle damage, loss of muscle K^+ and elevation of plasma CPK, and also directly correlated with uptake of ^{3}H-diphosphonate and with atomic-absorption-measured calcium uptake, that calcium accumulation presumably the basis for the diphosphonate localization. Our on-going autoradiography shows the uptake of ^{3}H-diphosphonate to be within muscle fibers, ones necrotic and even intact ones (presumably prenecrotic or perhaps reversibly injured) -- this correlates well (in histochemically-stained serial sections) with calcium accumulation within, and complete loss of phosphorylase/glycogen staining from, those fibers. In press is our report showing that calcium accumulation in damaged muscle fibers is also evident by light-and electronmicroscopy-histochemistry in various human disorders -- it is not disease-specific but it is a new way to identify damaged fibers, including minimally affected ones not identifiable by other methods. Our electronmicroscopy-histochemical study, using pyroantimonate, also in press, shows the calcium increase in non-specifically damaged fibers to be in mitochondria>sarcoplasmic reticulum>myofibrils. Nuclei (nucleoli and heterochromatin) are also calciumed. This pattern of organellar calciuming is the same one we find when normal intact muscle is soaked in a high-calcium solution.

From all of these studies we have formulated a new hypothesis covering molecular aspects of muscle fiber damage, as follows: (a) any damage to the plasmalemma -- be it, as we have demonstrated, exogenous (e.g., trauma, ischemia), endogenous deficiency of ATP-generating energy-supply (e.g., phosphorylase, phosphofructokinase, and carnitine-palmatyl-transferase deficiencies, experimental poisoning of glyceraldehyde-3-phosphate dehydrogenase with iodoacetate) or, conceivable but not tested, a direct primary or secondary plasmalemmal defect -- can break the normal plasmalemmal barrier to calcium (Ca_o^{++} / Ca_i^{++} is normally about 10,000-20,000/1); (b) the influxing Ca^{++} is taken up, as we demonstrated, by those organelles known to normally have the greatest capacity to do so, i.e., mitochondria>SR>myofibrils, and nuclei; (c) this heavy calcium accumulation eventually can damage organellar function -- e.g., a high Ca^{++} is known to reduce mitochondrial ATP synthesis, which presumably would lead to further impairment of the plasmalemmal barrier and increasing damage of the muscle fiber, and it also causes myofibrillar contracture and activates proteolytic processes, and may affect DNA and RNA; (d) thus, excess calcium within muscle (or other) cells may be the "ultimate molecular assassin", pushing damaged muscle fibers past their point of no return; (e) in contrast to the lethal cascade of events proposed to result from unrestricted or marked calcium ingress, if the increase of intracellular calcium is only slight and restricted, it might be relatively benign, and might actually be the spark for repair/regeneration or hypertrophy of muscle fibers, perhaps by displacing histones or other suppressor proteins to activate (derepress) DNA-RNA-transcription and hence RNA-protein translation systems in cells destined to survive, and it might also be the spark for cellular proliferation through activation (derepression) of DNA-replicating systems in minimally injured resting satellite cells, the cells destined to repopulate the tissue with myoblasts for reconstitution of muscle fibers.

Our theoretical and experimental data prompted our finding that computer-analyzed patient-scanning with ^{99m}Tc-diphosphonate can be used to detect abnormal uptake, presumably based on abnormal accumulations of calcium, in various disorders of muscle (with NM, CC). There is: (a) greatest uptake in the polymyositis/dermatomyositis complex (probably related to damaged muscle fibers and enhanced because of diffuse radiologically-invisible but histochemically and electromicroscopically-evident calcium in connective tissue of muscle and subcutaneous regions), in the presence of high, moderate or normal serum CPK; (b) moderate uptake in DMD patients, who have constantly very high serum CPK; (c) normal or minimally increased uptake in neurogenic involvement; (d) very high uptake in phosphorylase-deficiency and phosphofructokinase-deficiency muscle acutely injured by ischemic exercise and in muscle acutely injured by trauma (viz., at the site of muscle biopsy).

We are reporting that the histochemically "dark" muscle fibers characteristic of DMD are artifactually contracted and contain excess calcium, probably having incurred plasmalemmal injury during biopsy because of their abnormal fragility; such fragility we suggest is characteristic of DMD fibers. There are also more non-dark fibers calciumed in DMD than other myopathies, probably representing fragile fibers developing plasmalemmal injury in vivo as a reflection of the basic disease process. Other muscle-fiber markers of plasmalemmal injury we are studying are accumulated normally-barriered extracellular proteins, e.g., hemopexin, immunoglobulins and albumin.

A detailed study of mechanisms of muscle-cell injury in relation to Duchenne dystrophy is in press, or is a survey chapter (>500 references) of the biology of the muscle cell in relation to myopathies.

Other myopathies. Tissue culture of human muscle biopsies provides living muscle fibers growing free of all neural, vascular, and humoral factors present in the patients. After we developed a new program (reported two years ago) for achieving abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, and for precisely selecting certain fibers for enzyme-histochemistry, immunohistochemistry, EM, or EM-histochemistry, we have turned our attention to culturing the biochemically and the morphologically distinct myopathies.

In adult-onset acid maltase deficiency we earlier reported in one patient the first "reincarnation" in cultured muscle fibers of the biochemical and morphological defects characteristic of the disease, i.e., the first proof of endogenous myopathos of a muscular dystrophy, -- the muscle fibers in the biopsy and the ones newly grown in culture were identical by histology (vacuoles), histochemistry (acid phosphatase high in the vacuolar and prevacuolar regions), electronmicroscopy (glycogen accumulated in lysosomes) and biochemistry (with Columbia) (absent acid maltase by the natural-substrate method, 5-10% of normal acid maltase by the artifical substrate method, normal neutral maltase), normal kinetics of neutral and the trace-amount acid maltase, elevated acid phosphatase. That provided a new test system for manipulations directed toward the treatment or prevention of muscle-fiber damage in this disease without inducing any risk to the patient. Now we have shown similar reincarnation of the severe biochemical and morophologic defects in 4 additional chronic-infantile and 3 adult cases of acid-maltase deficiency, and partial defects in 3 heterozygotes. In the patients, a medium-chain-triglyceride ketogenic diet produced some subjective improvement -- this will be further evaluated.

We recently reincarnated a second metabolically distinct myopathy, phosphofructokinase (PFK) deficiency. Extremely reduced levels of that enzyme were found in muscle fibers cultured from a patient with a new syndrome of combined muscle and erythrocyte PFK deficiency and hypolipoproteinemia (with CP-CC and Columbia). Growth of that muscle in culture was enhanced by β-hydroxybutyrate added to the medium, a ketone-body providing one of the alternate energy sources which that muscle must be depending on since its glucose-utilization pathway is defective. In the patient, forearm ischemic exercise was shown to produce electrically-silent contracture, preferential damage to type-II muscle fibers, (calcium accumulation when mild and frank necrosis when severe) and positive ^{99m}Tc-diphosphonate scan.

In phosphorylase deficiency, a third (actually our first tissue-culture) glycogenolytic-enzyme defect of muscle, we have in 3 additional cases confirmed our previous finding that the enzymatic absence from the muscle fibers is "cured" with their growth in culture (i.e., a remarkable and unexpected recovery of phosphorylase activity is seen in fibers in the regenerative state in culture and in vivo. We have also now shown that phosphorylase is normal muscle phosphorylase (not a genetically different type) by histochemical staining of isozyme elctrofocused gels, thereby demonstrating a true rejuvenation of an enzyme genetically programmed ultimately to be deficient in mature fibers (with Hopital des Enfants Malades, Paris). The therapeutic thrust needed now is to provoke maintenance of that phosphorylase in the mature fibers of the patient.

In two phosphorylase-deficiency patients, utilizing customized treadmill exercise to achieve reproducible serum CPK rises and muscle cramps under standard conditions, we found that i.v. triglycerides prevented the CPK rise but not the cramps whereas glucose-insulin prevented both. In one patient, an oral medium-chain-triglyceride ketogenic diet for two weeks also prevented the exercise-induced CPK rise. Thus acute-intravenous and chronic-oral triglyceride therapy can prevent exercise-induced rhabdomyolysis, reflecting plasmalemmal breakdown from failure of ATP supply (since it did not mitigate the cramps as did glucose-insulin, different mechanisms must be involved).

Because we have previously shown in phosphorylase-deficiency patients the presence of a small amount of phosphorylase in regenerating fibers and because phosphorylase is activitable via cAMP, we are seeking a beneficial effect of a phosphodiesterase-inhibitor.

A new diagnostic test has been introduced and found positive in muscle phosphorylase deficiency (many other neuromuscular disorders are negative): ^{99m}Tc-diphosphonate scanning shows increase of calcium in forearm muscle 24 hrs after the standard diagnostic forearm ischemic-exercise test (FIET), and this uptake approximately parallels serum CPK elevations. Correlated histochemical and our new autoradiographic technique for localizing the clinically-administered gamma-emitting ^{99m}Tc-disphosphonate documented uptake of the tracer into injured, calciumed muscle fibers, and they showed preferential injury of type-II muscle fibers after FIET in muscle-phosphorylase deficiency. (This is part of our effort to develop techniques for doing autoradiography of patient biopsy samples following injection of some of the short-lived gamma-emitting radionuclides used for patient-scanning, to establish direct scanning-histoautoradiographic correlations.)

Single-fiber EMG of the patients during and after FIET established electrical failure of all motor units during FIET, and later no abnormal jitter and no motor-unit densification (i.e., no foreign reinnervation of type-I muscle fibers) -- thus although both fiber types fail during FIET, the I-fibers were not remarkably damaged (perhaps because of greater supplies of, and ability to use, endogenous non-glycolytic substrates and myoglobin-associated O_2, or could their axons have "protectively" shut off. These findings provide a basis for formulating alternate-pathway-substrate therapy in MPD.

A new model of defects in muscle glycogenolysis/glycolysis was produced using iodoacetate (which inhibits glyceraldehyde-3-phosphate dehydrogenase) intra-aortically plus repetitive sciatic-nerve stimulation: there was an electrically-silent contracture and 24 hr later a positive ^{99m}Tc-disphosphonate scintiscan, and low-oxidative type-II muscle fibers were preferentially injured and with histochemically evident calcium-uptake early and later frank necrosis. This model makes possible controlled analyses of the stages of intrinsic energy-depletion muscle injury, and subsequent repair, as well as evaluation of various alternate-pathway therapies applicable to human GG/Gly defects, e.g., phosphorylase or phosphofructokinase deficiency.

A new syndrome of fasting-induced (presumably) fatal rhabdomyolysis has been found. The biochemical cause is unknown -- none of the known biochemical defects of muscle is present and anaerobic glycolysis is intact. Lipid droplets were slightly increased in muscle fibers and biochemically carnitine palmatyl transferase was 2X normal -- an ATP-generating lipid-metabolism-defect is suspected (with Columbia).

Because carnitine is essential for the transport of long-chain fatty-acids into muscle mitochondria for oxidation and because carnitine deficiency causes a progressive myopathy, we are now studying autoradiographically the time-course uptake and localization of ^{3}H-carnitine into specific muscle-fiber types of animals and of tissue-cultured muscle. We will also develop a method for the same studies of patient muscle-biopsies *in vitro*.

A morphologically characteristic defect, "cabbage bodies" (multilamilated material in lysosomes) in muscle fibers of a patient with a chronic myopathy, were reproduced in his cultured muscle fibers. Although the biochemical defect is not known, both the biopsied and cultured muscle had elevated acid phosphatase, suggesting the patient has a defect of a yet-unpinpointed lysosomal hydrolytic enzyme (with Hopital des Enfants Malades, Paris). In that and our other cultures of human and animal muscle we are assaying 12 different lysosomal enzymes to search for defects thereof (with Hopital des Enfants Malades, Paris).

"Ragged-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical manifestation in limb muscles of the heterogeneous syndrome of oculocraniosomatic neuromuscular disorder with ragged-red fibers (OCSNMD-RR) (the patients often having lacticacidosis and often ophthalmoplegia. Some patients have a syndrome of small stature, seizures, mental impairment, and lacticacidosis. In limb muscle cultured from such patients, most of the mitochondrial changes, including increased number and greatly increased size of mitochondria, wide distorted "twisted-ribbon" cristae, and "mushy" inclusion material, have been re-incarnated, although the mitochondrial crystal-like inclusions have not yet been (perhaps they take longer to develop). The same changes were produced in normal human muscle cultures after 2 days exposure to dinitrophenol, and the ragged-red-fiber cultured muscle was extremely susceptible to worsening of the in vitro changes by dinitrophenol. This demonstrates a mitochondrial defect which is reproducible in cultured muscle fibers and provides a test-system for seeking a possible genetic or occult-infectious basis. In the original biopsies and in the cultures the mushy and crystalline inclusions lacked cytochrome oxidase staining by our EM-cytochemistry.

Clinically, some OCSNMD-RR patients with ophthalmoplegia have cerebellar ataxia, mental impairment, some denervation evident in muscle biopsy, and spinal-fluid protein increase -- of such patients, some have by CAT-scan a small brain-stem (shown by enlarged 4th ventricle and pre-pontine cisterns) and a decreased attenuation coefficient of cerebral white-matter, changes absolutely correlated with electrophysiologically-determined delay of the bilateral late-response of the blink reflex (with SNB and EEG).

Soft-tissue scanning of several OCSNMD-RR patients with ^{99m}Tc-diphosphonate has been positive, and curious in view of their normal serum CPKs. By pyroantimonate EM-cytochemistry calcium was not increased in the abnormal mitochondria of ragged-red fibers.

In muscle cultured from a Dutch patient (with Wilhelmina Gasthuis) with X-linked recessive severe-infantile muscle-fiber-hypotrophy-and-central nuclei, there was very abnormal proliferation with bizarre nuclei -- so much so that from a cloned single myoblast a cell line was started and carried through 6 passages, until it was lost because of incubator failure. Biopsies from the carriers will be used for a repeat attempt to develop a human muscle-cell line.

Abundant leptomeres were found in muscle cultured from 5 patients with acid-maltase deficiency and 1 with autophagic vacuolar myopathy but not in our other cultured normal or pathologic muscle. Similar abundant leptomeres were reproduced in normal human muscle fiber cultures exposed to dinitrophenol.

In tissue cultures of "normal" chick embryo skeletal muscle we were able to induce certain bizarre changes of mitochondrial morphology by pulses of dinitrophenol (DNP), but did not produce all the mitochondrial changes occurring in the human "ragged-red" fibers. In the course of that study we found, and reported, that DNP markedly promoted the detectability of avian oncornavirus in all such "normal" chick-muscle cultures -- virus was evident morphologically (C-particles) and by complement-fixation titers (COFAL). That demonstrated that the "normal" chick embryo muscle cultures so frequently used by various investigators for physiologic, biochemical, immunologic and developmental studies are actually virally-contaminated test objects. The question of whether that oncornavirus has a "normal" role in development of "normal" chick muscle *in vivo* or *in vitro* was raised by that study. Our finding the C-particles exclusively in dilations attributed to T-tubules raises the intriguing possibility that T-tubules might be the aqueducts of virus infestation of or shedding from muscle fibers. The question of viruses harbored in mitochondria (as "mitochondriophages", analogous to bacteriophages) is raised by the C-particles being provoked by DNP, an uncoupler of mitochondria-oxidative phosphorylation. Finally, we have raised the speculation that such "mitochondriophages" might be responsible for the mitochondrial changes in the ragged-red-fiber diseases.

Pyruvate kinase assayed in cultured human muscle by electrofocusing and histochemical staining was shown for the first time to have fetal isozymes (with Hopital des Enfants Malades, Paris).

In biopsies of 5 patients with sporadic chronic myopathies we have by electronmicroscopy found under the plasmalemma long strands suggesting viral material. Its nature and significance are being studied by cytochemical stains for DNA and RNA at the ultrastructural level, assays of reverse transcriptase, and attempts to reproduce it in culture. Our series of 12 patients with idiopathic vacuolar myopathy, some reincarnated in culture, is being reviewed in detail for publication (with Wilhelmina Gasthuis).

Neuromuscular disorders of uncertain classification. We continue to study the selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype IIB fibers, which we have shown to be the basis of cachectic atrophy accompanying cancer and other chronic debilitating disorders. Evaluation of the cause of type-II fiber atrophy in cancer patients, theoretical mechanisms of which we published last year, is important because this "remote-effect" muscle weakness is often the most crippling aspect of cancer -- if the molecular mechanism can be discovered it might be treatable independently of treatment and response of the cancer itself. And improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy.

Malignant hyperthermia-rigidity (MHR) is a syndrome, 70% fatal, of acute rise of body temperature and muscle rigidity during general anaesthesia. A number of the patients have underlying not-well-defined neuromuscular disorders. In one MHR patient, and his father, we are reporting central-core disease (CCD), with its typical type-I muscle-fiber predominance (with Children's Hospital, D.C.). Because we have another family with CCD and MHR and there are in the literature two other such families, we have issued a caution to all our CCD patients and their physicians regarding the possibility of MHR during general anaesthesia. The mechanism(s) of the attack of MHR is not known. It appears that there is an excess of free intracellular calcium in the muscle fiber, which we propose might be due to an effect of the anaesthetic or muscle-relaxant agent on the calcium-barrier function of the plasmalemma (v.s.) (rather than the SR as proposed by others). We are now investigating why central core disease, which we have earlier postulated to be due to a pre-natal monophasic neuropathy mainly affecting the type-II units, should predispose to the development of MHR. We are also working on new tests for specific clinical and muscle-biopsy diagnostic tests to identify patients predisposed to develop MHR.

Cross category and basic aspects. None of the eye muscle fiber-types is histochemically exactly like a limb-muscle fiber-type. Being published is our demonstration of their normal histochemical patterns in Rhesus monkey and identification of 3 types, "fine", "granular" and "coarse". The first two have one endplate per fiber and probably are different types of twitch fibers; the last has multiple endplates and may be a tonic fiber. Following denervation the first two developed diffuse extrajunctional acetylcholine receptors but the coarse fibers did not -- no fibers were positive beyond 13 weeks post-denervation. To secure more adequate samples with less discomfort a new needle-biopsy device has been designed. (Needle muscle biopsies are satisfactory when only a small amount of tissue is required for non-histochemical purposes, e.g., for tissue-culture or biochemistry.)

Mammalian plasmalemmal membranes have received most of our biochemical attention, through use of a battery of techniques. Results of the following studies are now being published or have been presented at meetings. Pure fractions of rat plasmalemma were obtained, and the methods perfected so that adequate quantities of plasmalemmal-membrane can from normal human muscle be obtained from limb amputation or radical mastectomy, and even from pathologic muscle biopsies for limited studies. With the plasmalemmal fractions and subfractions, methods have been established for studying acetylcholine receptor, acetylcholinesterase, Na^+-K^+ ATPase (Na^+-stimulated phosphorylation), divalent-cation (viz., Ca^{++}) binding/transport, Ca^{++}-stimulated ATPase, adenylcyclase, guanylate cyclase and catecholamine receptors. Some of these have been studied in sarcoplasmic reticulum (SR) fractions as well. Elucidated have been detailed properties of: (a) the adenylcyclase (i.e., fractional localization, kinetics, catecholamine-activation [the plasmalemmal AC was demonstrated to be a β_2 adrenergic receptor], guanylyl-inidodiphosphate activation, insulin and glucagon inhibition, all in animal "red" cf. "white" muscle and also the responses to denervation in "red" cf. "white" muscle; (b) differences between the catecholamine-sensitive adenylcyclase and β-receptor-binding (by use of ^3H-dihydroalprenol); (c) in human muscle the catecholamine-sensitive and GTP-stimulated adenylcyclase is predominately localized in the plasmalemmal fraction; (d) the human plasmalemmal adenylcylcase, on the basis of various agonist and blocker effects, showing that it is closely associated with β_2-adrenergic receptors, that its catecholamine-responsivity (but not its basal activity) is enhanced by GTP (and to a lesser extent by ITP), and that GMP-PNP stimulated both the β-catacholamine-responsive and basal adenylcyclase, and that both GTP and GMP-PNP act at the same site, indicating guanyl nucleotides are potent regulators of this plasmalemmal enzyme; (e) adenylcyclase (AC) in regenerating animal muscle, indicating that 5 days after crush the basal and isoproterenol-stimulated AC was decreased 30% in the plasmalemmal fraction and enhanced

40-60% in the SR and mitochondrial fractions -- since ^{45}Ca-uptake by the plasmalemma was increased 60% (and unaffected in the SR fraction) and Ca^{++} can inhibit AC, it is possible that the decreased AC in the plasmalemma in the in vitro assay was due to calcium accumulation; (f) guanylate cyclase (GC) re. subcellular distribution (highest in plasmalemma), kinetic properties, ionic requirements, molecular weight (about 300,000 for the plasmalemmal GC and 90,000 for the soluble form), and response to denervation (2-fold increase by 2 weeks of plasmalemmal and soluble GC, with unchanged kinetic and chromatographic properties, accompanied by 50% increase of cGMP-PDE activity; (g) the plasmalemmal protein phosphorylation (i.e., Na$^+$ enhancement blocked by K$^+$, phosphorprotein state suggesting an acylphosphate bond, high turnover-rate suggesting it is a functional intermediate of Na$^+$K$^+$ ATPase, and molecular weight); (h) the effect of denervation on this transport-ATPase system, (i) the Ca^{++} uptake and release by human SR (i.e., by use of specific antibodies made against SR which block the Ca^{++} uptake and inhibit adenylcyclase but do not affect ATPase activity, suggesting different localization of these functions within the SR; (j) the role of bound-calcium in the regulation of Ca^{++} transport by SR; and (k) physicochemical properties of ACh-receptor cf. acetylcholinesterase. These assays are now being used to elucidate biochemical defects of plasmalemmal or SR function in muscle biopsies from patients with various neuromuscular disorders (see our Episodic-Weakness-and-Myotonia project).

Insulin-receptor-function analysis of our cultured chick muscle fibers, intended as the first step toward studies of diabetic neuropathy patients (with Georgetown U.), showed that the uninnervated embryoid cultured fibers had no functional insulin receptors, in contrast to mature innervated fibers in vivo.

We have established microtechniques for cAMP and cGMP phosphodiesterases (PDEs), and are applying them to a portion of our patient muscle biopsies, and patient platelets. We have also applied it to cultured chick muscle and demonstrated a strong in vitro inhibitory effect on cAMP-PDE of phthalazinol. We are also studying the effect of dibutyryl-cAMP and phthalazinol on growth and maturation of human and animal muscle fibers in culture.

Acetylcholinesterase (AChE) subcellular distribution and properties showed: (a) majority present extrajunctionally, mostly in microsomes-SR but some in the soluble phase; (b) after denervation, the early AChE reduction is mainly in the microsomal-SR fractions; (c) the 16-S form is restricted to the neuromuscular junction (per other), while the microsomal-SR fraction had mainly 4-S (and this was the AChE abruptly reduced after denervation) and the plasmalemma had only 10-S and 16-S. Synthesis, degradation and release of the different molecular forms of AChE will now be studied in normal and various pathologic circumstances.

Myasthenia gravis (MG) is an acquired disorder affecting transmission at the neuromuscular junction, mainly in adults and older children. The primary cause is not known but the pathogenic mechanism is considered to be "autoimmune" or "dysimmune". Untreated patients usually are seriously handicapped and many die. Palliative treatment with anticholinesterases and antipathogenic treatment consisting of thymectomy, ACTH and, most recently, prednisone has helped considerably but much disability, some fatality, and drug side-effects still occur. Our studies involve improving diagnostic methods, seeking the cause and analyzing the pathogenic mechanisms, and improving methods of treatment. We also seek fuller understanding of the basic biologic and pathologic responses of the neuromuscular junction.

Two years ago we reported the first identification of a factor, an IgG, in the sera of MG patients which blocks binding of alpha-bungarotoxin (αBT) to the human junctional acetylcholine receptor (AChR) at the normal neuromuscular junction (41% of MG patients) and extrajunctional AChR of denervated human fibers (72% of MG patients, including all who had a thymoma), i.e., an "antireceptor antibody". We next reported that all MG patients having an IgG "antimuscle antibody" (first found by others) also had the blocking factor (although only half having the latter had the former) (the only discordant finding being in one non-myasthenic polymyositis patient with thymoma who had antimuscle antibody but no detectable blocking factor) and suggested that these may be the same antibody or, if different, virtually always co-produced. Our junctional localization of the blocking factor put it in the correct position to impair neuromuscular transmission and cause the weakness of MG. MG sera positive for blocking-factor were shown to block the binding of αBT to the diffuse extrajunctional receptors of aneurally cultured human, rat, and chick muscle fibers (additional studies of extrajunctional ACh receptors are detailed in our ALS Project).

On the basis of those studies and our EAMG studies (v.i.) we have now hypothesized the following: (a) two subsets of acetylcholine receptor (AChR), a "junctional" (J) and an "extrajunctional" (E) form; (b) both J and E AChR occur at the neuromuscular junction but extrajunctionally only E-AChR is present; (c) the IgG blocking factor in human MG is directed mainly against the E type of AChR while that of the EAMG model we worked with is mainly against the J-AChR; (d) the diffuse AChR of thymic epithelial cells, v.i., may be E-type.

From our work with the induced autoimmune model (rabbits injected with electric-fish AChR), originated by others, of experimental allergic MG (EAMG) (with U. Maryland) we have in-press our demonstration of: (a) binding of that rabbit sera to human neuromuscular junctions but not to extrajunctional receptor of denervated fibers at light-microscopic resolutions; that junctional binding of EAMG sera was not blocked by blocking-factor-positive (or negative) MG sera, only partially blocked by αBT, and not blocked by carbamylcholine, decamethonium or tubocurarine; (b) binding of it by electronmicroscopic resolution to the plasmalemma diffusely in rat and chick muscle fibers in tissue culture; (c) binding of that rabbit sera to the original antigen in a radioimmunoassay we developed (with IB), but no binding of blocking-factor-positive (or negative) MG sera to that antigen; (d) similarities but also distinct differences of the model with human MG, indicating it is not a perfect model of the latter although it could still be a model-in-principle. We are now exploring for blocking factor in another potential MG-model, the rhesus monkey with MG-like thymus hyperplasia induced by penicillamine.

Our electronmiscroscopic-immunohistochemistry demonstrated junctional αBT bindings presumably localizing AChR, at both the muscle post-synaptic plasmalemma and, to a lesser extent, at the axonal pre-synaptic plasmalemma, indicating that the IgG blocking factor, if indeed pathogenic, would act both post- and pre-synaptically in MG. Data from other types of studies by others support a pre- as well as a post-synaptic locus of AChR in normal neuromuscular junctions. But because the pre-synaptic localization is considered by others to be a diffusion artifact, current experiments are directed toward study αBT-binding to motor axonal terminals in isolation.

B-lymphocytes presumably are the cells making the junctionotoxic IgG in MG, but why? That the thymus is involved in many cases of MG is well known, but its role is not. In the thymus we have now demonstrated that the epithelial cells, typically hyperplastic in MG, all contain AChR demonstrable histochemically by αBT binding (with IB and Washington Univ.). This fulfills a step in our earlier hypothesis that the mechanism of MG might be an alteration of thymic epithelial cells e.g., by an exogenous virus, making them "foreign", in response to which B-cells make anti-AChR antibody (and perhaps T-lymphocytes also react) that co-reacts with junctional AChR to cause paralysis. Because the thymic epithelial cell is by others, considered pluripotential, this same hypothetical pathogenic mechanism could apply to other dysimmune diseases in respect to other thymic epithelial cell molecules altered to become antigen. Indeed, the multifactorial nature of the dysimmune in the sera of a significant number of patients (with NIAMDD). We have also found serum hemopexin inexplicably increased in MG patients; since there is no known leakage of a heme-containing component in MG, we are exploring whether this is another, previously unrecognized, dysimmune manifestation. We are analyzing the molecular structure of the hemopexin produced in MG for comparison with that produced in leaky-muscle-cell myopathies.

Our electronmicroscopic studies of human thymic epithelial cells from myasthenia gravis patients, directly (regular and pyroantimonate-stained tissue) and after being grown in culture, showed desmosomes and tonofibrils typical of epithelial cells and no muscle-cell like myofibrils contrary to what has been reported by others in cultures of normal animal thymuses (which might have been contaminated with non-thymus muscle cells during tissue removal).

A lymphocyte reaction within the thymus to the proposed "foreignness" of thymic epithelial cells could be of either B- or T-lymphocytes, or both. Our counts of lymphocyte types in fresh MG thymuses as removed at therapeutic thymectomy (histologically lymphoid-hyperplastic) compared with normal thymus demonstrated a slightly increased percent B-lymphocytes, suggesting a reactive increase of them there. Our one-way and two-way mixed reactions with thymic and peripherial-blood lymphocytes from MG patients, compared with control patients, were not abnormal, neither showing evidence of self-stimulation either way nor altered peripheral T-lymphocyte modulation by thymic-lymphocytes (thymic epithelial cells were probably not in our thymic-lymphocyte fractions but may have contaminated those of other studies).

Thymectomy is an established treatment for MG. Our surgical preference continues to be transverse sternal-splitting for adequate removal of thymic tissue (with NIHL) -- our mortality is zero, morbidity low and therapeutic response good to excellent, but often not permanent. More detailed evaluation of our 50 thymectomies since 1968 is underway, correlated with the thymic pathology (with Harvard).

We have now demonstrated that repeat thymectomy can be remarkably beneficial in patients previously improved who subsequently exacerbate and do not respond to medical management. In a number of those patients as well as primary thymectomy patients, we have found positive ^{67}gallium scans -- our thymectomy series is now being correlated with the thymic pathology (with N. M. CC and Harvard). Further, cellular localization of the ^{67}gallium is being done by autoradiography of the excised thymus -- preliminary results suggest the accumulation is in the active lymphoid follicles.

Abnormal lymphocyte function is the pathogenic step presumably suppressed by corticosteroid treatment of MG. Confirmed and adopted by most other physicians has been the treatment we introduced to MG, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred). In our own series it continues to be extremely beneficial in the majority of cases, 48 of 52, and for as long as 11 years in a child and 7 years in an adult. Responding best are the older-onset patients, especially the older males. Our 4 non-responders were females, 3 in the menstruating age group. Importantly, though, we have reported that none of our responding patients has become absolved of his requirement for prednisone, even after a gradual tapering of the dose. For example, two patients have exacerbated 3 months after stopping a 5 mg q.o.d. dose (this is about the length of time taken for resumption of synthesis of significant abnormal IgG levels as measured by others in another dysimmune disease). We have modified the treatment slightly by giving, to patients not simultaneously taking anticholinesterase drugs, the single-dose 100 mg prednisone daily for the initial 2-4 weeks before converting to the alternate-day schedule, apparently resulting in more rapid improvement. Fragile patients are not taken off their anticholinesterases but in them prednisone is given in a gradually incrementing single-dose-daily schedule beginning with 10-20 mg. Regarding combining anticholinesterase and prednisone, we reported that low doses of one can be combined with the other advantageously -- but that patients taking both drugs sometimes have a more "brittle" myasthenia and must be watched carefully for ingravescent overdosage by the anticholinesterase. However, because neither anticholinesterase nor prednisone treatment is either curative or completely suppressive, more details of the pathokinesis are needed (v.s.).

Some of our patients have required much higher prednisone doses than 100 mg q.o.d., or even q.d., dosages arrived at empirically in each patient after a period of time. We would like to be able to give a single test-dose and measure parameters of immunosuppression to immediately establish the minimum effective dosage needed, since overdosage, and even therapeutic dosage, produces side-effects of various degrees. Accordingly, the effect of the HSDAD-prednisone treatment on lymphocytes was measured over a 48-hour cycle in a number of MG and other patients, and the results presented. At 6 hrs. after the 8:00 a.m. prednisone dose there is marked depression of

T-lymphocyte counts and lymphocyte responses to T-lymphocyte mitogens, and a lesser effect on B-lymphocytes and response to B-lymphocyte mitogens, and there was return of these measurable effects to normal by 24 hrs. after the dose (yet clinically the prednisone has a cumulative beneficial effect over weeks and months). The effects were approximately dose-dependent, establishing that these parameters might answer the original need, but the considerable individual-patient variability means that further precision must be gained.

Our recently-published multistep hypothesis of the pathogenesis of MG, revised on the basis of the blocking factor, bears a striking analogy to the scenario of Verdi's Rigoletto. We therefore term the presumably-detrimental blocking factor a "Sparafucile" molecule after Verdi's hired assassin who without malice killed the unintended victim, in fact, killed the beloved daughter, Gilda (the NMJ-located AChR receptor in MG) of Rigoletto (? malfunctioning helper or suppressor T-lymphocytes), who planned the assassination to be of the Duke of Mantua (? AChR-containing "foreigned" thymic epithelial cell). It is evident from further analysis of the scenario that the answer to MG will only come with the identification and treatment or prevention of Count Monterone's curse (? an environmental factor, e.g., virus).

Our current experience with congenital-persistent myasthenia gravis indicates it is different from the juvenile-onset or adult-onset forms, and probably a different disease. These differences are being codified and will be reported.

The remarkable ancillary benefit that broad-aspect nursing can provide to an MG patient is repeatedly evidenced in our patients -- our multidimensional nursing care approach for myasthenics is available on videotape (with Nursing, CC) and available for general distribution -- this undoubtedly will help improve the care and perhaps save the lives of some myasthenic patients with serious disease, especially in hospitals not frequently caring for such patients. On our own Nursing Unit, MG has been designed as the disease selected for initial development of audit-criteria.

A new electrophysiologic defect of neuromuscular transmission has been found in a patient with a fatigue syndrome and chronic renal disease -- after a brief tetanus there is a diminished but broadened total-muscle action-potential which gradually recovers during rest or slow stimulation, and it is not ameliorated by edrophonium, guanidine, or Ca^{++}. The histochemistry of her muscle biopsy showed marked I-fiber type-predominance but no I-fiber-subtype-grouping, harmonizing with the single-fiber-EMG finding (with Uppsala) of no increased densification of the motor unit -- this was presented.

A new experimental model of neuromuscular junction transmission defect has been found after immunizing animals with peripheral nerve axoplasmic "soluble" protein (see our ALS Project).

The single-fiber EMG technique to study "jitter" and "blocking" phenomena and motor-unit densification, recently described by others, was set up during the visit of E. Stalberg -- it is serving to sharpen our diagnostic and investigative studies in MG and other neuromuscular-disease patients.

Periodic paralyses (PP) are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. Associated metabolic abnormalities are known but the actual pathogenic mechanisms are not. Standard palliative preventive therapy in the idiopathic hypokalemic form of PP is potassium, and more recently acetazolamide. Our studies involve improving diagnostic methods, seeking the causes and analyzing the pathogenic mechanisms, and improving methods of treatment.

In the hypokalemic form of PP, the treatment we introduced, long-term acetazolamide, has continued to be the best prophylactic agent both for preventing attacks and improving inter-attack weakness. It is now in the textbooks as such. Two of our patients have been treated successfully for more than 11 years. Renal calculi in one of 27 patients, possibly but not definitely related to the acetazolamide, have been the only suspected side-effect. Since muscle does not contain carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown.

Clinical muscle scanning for calcium with ^{99m}Tc-diphosphonate of 3 hypokalemic PP patients did not show any accumulation in muscle during or between attacks, nor any CPK fluctuation.

Myotonia is crippling to various degrees in myotonia congenita and paramyotonia congenita (inherited diseases of unknown cause). A controlled clinical trial showed moderate to remarkable benefit from acetazolamide in 8/10 patients who had failed to respond to, or had been intoxicated by, other anti-myotonia drugs. It remains the long-term treatment of choice in 6 of those patients. A controlled trial of lithium carbonate in one myotonia congenita patient showed decreased myotonia but only at very high levels ($Li > 1.8$ mEq/l).

Myotonic atrophy (myotonic "dystrophy") is an inherited multisystemic disease, with progressive wasting, of unknown pathogenesis. We have previously raised the possibility of at least a partially neurogenic aspect. With our new concept of "myogenous de-innervation", we have now extended that hyothesis to include a possible myogenous muscle plasmalemmal non-receptivity to neural short- and long-term trophic influences.

The model of 20,25 diazacholesterol-induced myotonia of animals was used to evaluate myotonic phenomena. Muscle of the markedly myotonic animals showed: (a) no histochemical changes, (b) no blockage in vivo of the myotonia by α-bungarotoxin, d-tubocurarine, succinylcholine or atropine in contrast to blockage of sciatic-neurectomy-induced fibrillations by all four drugs (and blockage of both myotonia and fibrillations by procaine, tetrodotoxin, KCl or ischemia and of neither by pyridostigmin). Our results demonstrate a major difference, and several similarities, between fibrillation and myotonic discharges -- it is possible to hypothesize that fibrillations and DAC-induced myotonia are mediated through mechanisms involving ionic channels, that both can be produced by activation of junctional/nonjunctional AChRs (or some mechanism coupled to those receptors), but that an unfettered αBT binding portion of the AChR molecule, and an unblocked atropine-binding site, are obligatory only for production of fibrillations. The subcellular localization of tritiated 20,25 diazacholesterol (DAC) is now being analyzed by autoradiography, electronmicroscopic-autoradiography and biochemistry of muscle cell fractions. This, and our showing lack of αBT binding histochemically to muscle fibers in human myotonic diseases, indicate essential differences between myotonic and fibrillating plasmalemmas. Presented was that DAC --induced-myotonic-rat-muscle plasmalemma had 40-60% lower levels of basal and fluoride- and catecholamine-stimulated adenylcyclase activity. Similarly, a 30-60% adenylcyclase decrease was found in 5 patients with myotonic atrophy. These changes could be due to confirmational changes of the enzyme molecule or an altered sterol composition of the plasmalemma.

We confirmed histochemically the finding of an increased number of intrafusal muscle fibers in muscle spindles of myotonic atrophy patients. Our new finding was that muscle spindles are normal in myotonic congenita patients and in rats rendered chronically myotonic with 20,25 diazacholesterol. Therefore, spindle abnormalities in myotonic atrophy perhaps are due to fusimotor deinnervation (? neurogenous myogenous deinnervation) rather than to myotonia per se; moreover, we have found similar spindle abnormalities in rat muscle spindles experimentally denervated (neurogenously).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01039-15 MN

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.6	4.3	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In amyotrophic lateral sclerosis (ALS) and other diseases affecting the lower motor neurons, including peripheral neuropathies and some spinocerebellar degenerations, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human pathophysiologic states.

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Project Description:

Objectives: In amyotrophic lateral sclerosis (ALS) and other diseases affecting the lower motor neurons, including peripheral neuropathies and some spinocerebellar degenerations, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human pathophysiologic states.

Methods Employed: A variety of techniques, encompassing histochemistry, biochemistry, autoradiography, radionuclide scanning, electrophysiology, electronmicroscopy, immunology and tissue culture are applied to patients with the various diseases covered in this category and to induce animal-models thereof. Conducted were double-blind, single-blind and non-blinded therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us for quantitating isometric muscle tension.

Patient Material: Medical Neurology Branch patients and neurology consultation patients from the various services of the Clinical Center, who had diagnostic muscle biopsy and other diagnostic procedures.

Major Findings:

Amyotrophic Lateral Sclerosis (ALS): Previously we have reported a neurologic abnormality with certain features like ALS in many cases of primary and secondary hyperparathyroidism; we have also studied a few cases with both frank hyperparathyroidism and frank ALS. To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have studied various parameters of calcium homeostasis. Reduction in whole body retention of radioactive calcium (>80 retention tests) was seen in patients with neuromuscular disease compared to normal controls. Reduction in retention in ALS patients was more pronounced than in myopathy and neuropathy patients. That reduction was due in part to inactivity because paraplegia regardless of etiology gave reduction in whole-body retention. ALS patients who were not paraplegic still had significantly reduced whole-body retention. That decrease was not due to decreased 25-hydroxy vitamin D levels, nor to resistance to dihydrotachysterol because administration of the latter corrected the decreased retention. Serum parathyroid hormone concentrations, measured, were higher than normal in 40-50% of all patients with neuromuscular disease and similar in all subgroups: ALS, myopathy, neuropathy. Response to calcium infusion will now be evaluated to determine if there is an abnormal response to imposed serum calcium fluctuations, because we have found that absorption of ^{45}Ca from gut was enhanced in ALS patients compared to disease-controls, yet serum calcium was normal and not different from disease-controls.

Folate (oxidized and reduced forms) and B_{12} in CSF and serum in sporadic and familial ALS was measured and compared to values in neuropathy and myopathy (with Johns Hopkins and Bronx VAH) and no significant reductions found when age and sex-matched groups were compared. Thus the posterior column degeneration in familial forms (originally described from this Branch) is not directly due to local reduction of folate of B_{12} (however, interference with its action on cells could be occurring). Additional data suggests that probenecid does not cause rise in CSF folate in ALS patients.

In ALS patients, including those with a late-post-polio progressive muscular atrophy or myopathy syndrome, we continue to search for evidence of a viral cause in ALS sera, CSF and tissues, by profiling of viral anti-bodies (with Johns Hopkins and IDB), and various tissue-culture techniques (including activation and evaluation by morphologic, antigenic, and reverse-transcriptase assays). To date, no viral evidence has been found.

Study of 50 ALS patients, age and sex matched with neuropathy and myopathy disease-controls (with IDB and Johns Hopkins) show no difference in serum antibodies to polio types 1, 2, or 3 nor of CSF antibodies indicating no significant local CNS production of poliovirus antibody. One subgroup with rapidly progressive ALS showed CSF antibodies which cross-reacted with rubella antigen -- we will now do radioimmunoassay of antibody levels to rubella and arbovirus antigens.

CSF IgA was found increased in untreated ALS patients compared with no-neurologic-disease and neuropathy and myopathy disease-controls (with IB); serum IgA also was elevated in those patients compared to the above controls. We are now determining whether there is a specific component of local IgA production in CNS reflected in CSF. Serum and CSF dynamics of IgA metabolism will then be studied. In 12 ALS patients, oligoclonal bands were sought in the CSF, but none found (with IDB). HLA-typing has not revealed any predominant types in our ALS patients.

As an induced animal-model (with LNC and Johns Hopkins), Swiss mice acutely infected with poliomyelitis, which attacks anterior horn cells, are being studied to acertain whether the time-course of reduction of neurotransmitters, substrates high-energy intermediates, and cyclic nucleotides shows an early drop, e.g., of cGMP, in spinal cord antecedent to paralysis, attempting to determine the sequence of the virus-induced biochemical alterations. We will also compare controls ± cyclophosphamide to polioinfected ± cyclophosphamide animals to eliminate the role of inflammatory cells which might have elevated cGMP.

From our continuing experience with more than 550 patients with ALS or ALS-like syndromes we emphasize that (a) about 10% of ALS is chronic, surviving 5-15 years or more (and therefore giving false hope to experimental therapists if they are not aware of this subgroup), and (b) as many as 15% or so do not have ALS and some are treatable, identifiable by careful clinical and laboratory examinations -- e.g., a patient this year presenting as ALS had posterior lumbar diverticula with ankylosing spondylosis.

Two extensive chapters are about to be published, one on numerous aspects of the biology of the lower motor neurons as a basis for understanding and investigating diseases thereof (>1000 references), and the other on various aspects of the several motor neuron diseases (>300 references), as well as 3 chapters on other neuromusular diseases: central core disease, rod disease, and muscle fiber hypotrophies.

Our new concept, introduced two years ago, developed on the basis of our combined EMG and histochemistry studies, our open-biopsy-EMG technique, and on theoretical grounds, that an EMG pattern on voluntary effort of brief-duration small-amplitude overly-abundant motor-unit action potentials (BSAPs), sometimes polyphasic (BSAPPs) is just as possibly a reflection of neuropathic as of myopathic diseases (and we criticized the established but vacuous cliche' of "myopathic EMG" for that pattern) becoming more widely accepted.

Two years ago we introduced the technique of α-bungarotoxin αBT) staining to demonstrate neurogenously deinnervated muscle fibers by their diffuse plasmalemmal extrajunctional receptors. Then, last year, we evolved the new concept of "myogenous mal-innervation" (in press), based on our demonstration that: (a) diffuse extrajunctional ACh-receptors were present on "regenerating-degenerating" muscle fibers in myopathic biopsies of our patients, (b) human, rat, and chick embryo skeletal muscle grown in tissue culture without innervation (pre-innervated muscle) has diffuse extrajunctional ACh-receptors, and (c) in adult animals, segments of muscle fibers experimentally separated from their motor innervation point but remaining viable develop diffuse extrajunctional ACh-receptors. Thus we have formulated a "law of mal-innervation", since it becomes evident that the presence of diffuse extrajunctional AChR in muscle fiber plasmalemma is certainly a sign of mal-innervation of the muscle fiber but it is not pathogenesis-specific since it can be either a neurogenous or a myogenous de-innervation of dys-innervation, or a primary non-innervation. Experimental myogenous deinnervation, as we reported last year, results in diffuse extrajunctional AChR, with which we are now correlating the appearance of fibrillations. In the cultured non-innervated muscle fibers of chick, rat and human tissue, the αBT-demonstrated diffuse plasmalemmal acetylcholine receptors (AChRs) of single myoblasts through multinucleated well-differentiated contracting fibers did not appear electronmicroscopically to have "hot-spots", as reported by others from light-microscopic autoradiography, -- we suggested "hot-spots" may be artifacts based on subtle plasmalemmal folds.

Correlations of histochemistry and single-fiber-EMG (SFEMG) have been made in patients with various neuromuscular diseases. Ordinary neurogenous deinnervations have significant densification of motor-units (from collateral foreign reinnervation of orphaned muscle fibers) and fiber-subtype grouping histochemically. Chronic myopathies (Duchenne dystrophy, polymyositis/dermatomyositis, limb-girdle morphologically-non-specific myopathy syndrome) had mild densification by SFEMG but no definite subtype grouping histochemically, indicating a greater sensitivity of SFEMG. From study of 8 patients with muscle fiber-type predominance without major evidence of ordinary denervation (5 central core disease, 1 congenital rod disease, 1 type-I-fiber-hypotrophy-with-central-nuclei, 1 type-I-fiber-predominant unclassifiable neuromuscular disease) it became evident that, in a field of fiber-type predominance, subtype-grouping is requisite for a histological diagnosis of neurogenous deinnervation (although its lack does not, hypothetically exclude an abnormality of LMNs).

Biochemical aspects of neurogenously de-innervated muscle, such as increased guanylate cyclase activity, are in our myopathy project.

Animal muscle spindles exhibit variations in the histochemical profiles along the intrafusal muscle-fiber length -- e.g., with the myofibrillar ATPase reaction the nuclear-bag-2 fiber stains darkly in the spindle-capsular region but lightly in the spindle-polar region. The significance of this variability is not known -- it may relate to the complex sensory and polyneuronal motor innervation of the intrafusal fibers. A current study of human muscle is correlating spindle-fiber histochemistry and electronmicroscopy, using matched interrupted serial-sections along the whole fiber length of the same spindle for fresh-frozen histochemistry and EM. Experimental motor deinnervation and reinnervation of animal spindles did not result in evident histochemical changes in contrast to extrafusal fibers, suggesting possible specificity of motor-axonal reinnervation or a trophic role of sensory innervation.

In patients with scoliois, our continuing studies show a wide variety of neuromuscular diseases (by muscle biopsy histochemistry) associated with and probably causing scoliosis, most commonly some form of neurogenic muscular atrophy. Some of our scoliosis cases were previously considered "idiopathic", a group we are studying in more detail.

Polyneuropathy (Peripheral Neuropathy) (PN): The peripheral neuropathies comprise a group of disorders of various causes, more than half unknown. They always cause serious physical handicap sooner or later in the course of the disease, sometimes associated with intractable pain and ulceration and loss of feet and hands. Our studies seek to delineate the underlying causes and where possible develop a treatment. We also seek fuller understanding of the basic biology and pathologic responses of the lowor motor and sensory neurons and peripheral nerves.

In the majority of patients we see the PN is of undiscernable cause. Those which are non-familial we may treat with LT-HSDAD-prednisone, especially if less than 5-years duration postulating a dysimmune pathogenic mechanism. This year we have formally summarized our ongoing experience in 25 carefully-documented patients who have had good to outstanding success, most having been given-up on by others and some having come with diagnoses of non-treatable diseases (e.g., ALS or "Charcot-Marie-Tooth" disease). Long-term treatment is required -- too-rapid reduction of dosage too soon results in exacerbation. Excellent results have been sustained for as long as 12 years in an adult and 9 1/2 years in a child (who at age 21 is still regaining motor skills). Our correlative studies indicate that patients most likely to respond have the triad (i) being dysschwannian in type (slow nerve-concuction times, (ii) relapsing, (iii) with elevated CSF protein, but even some non-relapsing patients (i.e., 2 with progressive course > 1 yr) without slowed nerve conduction times and with normal CSF have responded to LT-HSDAD-Pred. Prednisone has a broader scope of responsitivity in this disease than previously believed. Virtually all our corticosteroid-responsive patients are cortiosteroid-dependent requiring 5-20 mg single-dose q.o.d. to prevent exacerbation.

We have studied the mechanisms of HSDAD-Pred anti-dysimmune effect on circulating blood lymphocytes (see Myasthenia Gravis project) and its effect on cerebrospinal-fluid (DSF) lymphocytes and immunoglobulins. We developed a method to identify T- and B-lymphocytes in CSF and found percentages normally are the same as in peripheral blood (72 and 16%). In 9 chronic idiopathic relapsing polyneuropathy patients, with 2-10X elevated CSF IgM and or G, studied longitudinally, HSDAD-Pred caused reduction in CSF T- and B- lymphocytes and IgM, IgG and IgA, and serum IgG but not IgM or IgA; thus the accompanying remarkable clinical improvement may have been by prednisone affecting lymphocyte numbers and Ig production (with 1B) directly within the CNS. Our current studies of the pathogenesis involve investigation of oligoclonal Ig bands in the CSF and serologic investigation of sera and CSF re. acute/chronic viral infections using antibodies to 17 agents.

An often undiagnosed cause of sensory-greater-than-motor neuropathy beginning in adulthood is "idiopathic" amyloidosis. We reported the combination of crystal violet stain with fresh-frozen sections of a muscle biopsy (proven by us by more than a decade of use) as the preferred method of diagnosing this disorder. We reported our most recent 10 cases of non-familial amyloid polyneuropathy, the onset we reported of which was in later adulthood (mean age 54); 8 were male. We emphasized that this disorder is associated with, and probably the result of, a plasma-cell dyscrasia, detectable in 8/10 of our patients as multiple myloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments (IgG-kappa >> IgG-lambda, IgM-lambda). We propose that the neuropathy is due to a systemic metabolic abnormality, possibly related to a circulating abnormal protein fragment (i.e., a "para-Sparafucile" phenomenon, v.s.), rather than to pressures from multifocal amyloid deposits of immunoglobulin fragments or ischemia. Our treating 6 amyloid patients with melphalan, an "anti-myeloma" agent, was not of obvious benefit. We have also reported a new application of 99^{m} Tc-diphosphonate clinical radioisotope scanning (with NM, CC), as a diagnostic technique for identifying amyloid in soft tissues -- highly suggestive of this diagnosis is (in the presence of a normal soft-tissue x-ray) a diffusely positive scan, presumably based on binding of the tracer to cations that are binding to anionic groups in amyloid fibrils, a mechanism supported by our published EM histochemical and autoradiographic demonstration of calcium localized to amyloid. It also diagnoses cardiac amyloidosis in patients with "idiopathic cardiomyopathy". When quantitated it can be used to follow effect of treatment.

We have now established combined studies of patient sural nerve biopsies in vitro -- including in vitro nerve conduction velocities of fast and slow fibers, teased fiber histochemistry, electronmicroscopy, and EM-histochemistry, allowing more precise and direct multidimensional analyses of the afflicted nerves in polyneuropathy patients. This has shown, for example, marked slowed conduction in small (C) fibers correlated with small-fiber pathology when clinical conduction times (which do not "see" C-fibers) were normal. We have also established tissue-culture growth of human schwann cells from pathologic human nerve biopsies -- this will provide opportunity to directly study the pathologic cell growing in vitro and dysschwannian neuropathies such as dysimmune ones (e.g. "Gullian-Barre"), in diabetes mellitus and in metachromatic leukodystrophy. We are attempting to develop immunochemical methods to selectively identify individual schwann cells in culture, and will attempt to clone and produce a cell line of some pathologic human schwann cells.

Anterograde axoplasmic transport of protein, glycoprotein and other substances from the neuron soma to the periphery is critical in maintaining integrity of the peripheral nerves. There is also a retrograde axonal transport, the normal role of which is uncertain but, we hypothesize, may serve for: (a) continuous monitoring by the soma of its distal self (e.g., regarding the necessity or not for axonal regeneration), (b) monitoring the external environment of its tip, and (c) acquisition of nutrients; it may also serve as the mechanism by which toxic substances are acquired (v.i.). We have published our autoradiographic studies (with Johns Hopkins) demonstrating in animals a rapid anterograde transportation to the axon synaptic terminal at the neuromuscular junction and accumulation there of large amounts of protein and glycoprotein synthesized in the lower motor neuron soma from precursors leucine and fucose injected into the ventral horn less than 24 hrs. earlier. This demonstrated that these rapidly transported axonal proteins and glycoproteins go rapidly to the geographic location where they can be used, hypothetically, for (a) remodeling of the axonal tips and (b) trophic influence on muscle -- both possibilities we are studying further. Combined electronmicroscopy and autoradiography demonstrated that ligature of the nerve interrupted both anterograde and retrograde rapid axonal transport, causing intra-axonal accumulation of masses of smooth vesicular membranes on both proximal and distal sides of the ligature and of substances labeled from precursors injected centrally or peripherally respectively. Those results suggested that fast anterograde and retrograde axonal transport are very similar processes carrying predominantly membranous organelles and constituting a system of bidirectional fast transport (which is interrupted focally by the nerve ligation). Further, pathologic axonal swellings (at the cut ends of axons) were interpreted as the consequence of the acute focal interruption of this rapid transport system. Thus studies of axonal transport provide a means for investigating the origin and fate of axonal organelles in pathologic process. Our autoradiographic studies have also shown that fast anterograde axonal transport provides a necessary contribution to motor nerve regeneration in experimentally sectioned nerves.

A new principle/model for inducing an experimental allergic neuropathy (EAN) in animals is being reported. It utilizes immunization with soluble nerve protein fraction (in contrast to lipid-associated protein of myelin used in previous EAN models). This represents a new potential model of some human dysimmune-dysneuronal peripheral neuropathies such as in some patients we have seen with prednisone-responsive neuropathies without demonstrable schwann-cell involvement. It also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and parainfectious encephalopathies. Since our EAN animals also have a component of blockade of neuromuscular transmission that is responsive to edrophonium, the model may have some relevance to myasthenia gravis or other disorders of the neuromuscular junction.

Central Nervous System Disorders: Of relevance to the group of spinocerebellar degenerations comprises diseases of various causes, a few known, most not, which always result in serious physical handicap sooner or later in the course of the disease, and sometimes mental deterioration. Our studies seek to delineate the underlying causes, where possible attempt to develop a treatment, and define basic cellular pathophysiologic mechanisms.

cAMP is thought by some to be a mediator of synaptic transmission of some systems in the cerebellum. Our newly developed histochemical technique for its synthesizing enzyme, adenylcyclase (AC), shows the greatest amount to be in cerebellar blood vessels, and of the neural-associated enzyme the greatest amount was in the basket-cell basket endings at the base of the Purkinje cell, not in the Purkinje-cell soma as previously supposed from tissue-slice biochemistry -- this necessitates modification of current hypotheses of Purkinje-cell function. It also shows significant AC in blood vessels, which would influence biochemical assays of homogenates of microdissected tissue samples.

In Cockayne's syndrome (cachetic dwarfism, various neurologic dysfunctions, cutaneous sunlight sensitivity, recessive inheritance), the syndrome of normal-pressure hydrocephalus was discovered in all 4 patients studied (with NCI).

Progressive spastic paraplegia is a progressively crippling disorder of children and adults. The causes are not known. In press is our identification of three unrelated patients with a syndrome of chronic adrenal insufficiency from infancy juvenile-onset of progressive spastic paraplegia and dysschwannian peripheral neuropathy, with normal intelligence (with NIAMDD). A single metabolic defect is postulated to underlie the abnormalities in the neural and adrenal tissues (? an adrenoleucodystrophy variant).

Opthalmo-neurology: We have shown that the various neuromuscular disorders affecting the eyes, assuming they are correctly reflected by their limb-muscle pathology, can be on a neuropathic or myopathic basis. They cause various degrees of handicaps. Our studies seek to delineate the underlying disorder, analyze the neuro-opthalmologic defect, and, if possible, seek methods of treatment. We also seek fuller understanding of the basic biology and pathologic responses of the eye neuromuscular apparatus. The commonest associated limb-muscle pathology of the progressive external opthalmoplegia syndrome (in our series of 46 patients, after myasthenia gravis and myotonic atrophy are excluded) is a syndrome characterized by "ragged-red" muscle fibers in limb muscles, whether or not the limbs themselves are weak. Those ragged-red fibers, containing several types of mitochondrial abnormalities, have been studied intensively (see our Myopathy project). Other limb muscle pathology associated with progressive external opthalmoplegia in our cases includes (i) a vacular myopathy-plus-neuropathy, (ii) type-I-fiber-hypotrophy-with-central-nuclei, (iii) only denervation, (iv) only morphologically nonspecific myopathy, and (v) type-II-fiber smallness. The histochemical fiber-typing of normal animal eye muscles and the different post-denervation response of extrajunctional receptor distribution of those fiber-types is in press (see Myopathies project). Abnormalities of eye movement in the spinocerebellar ataxias and in myasthenia gravis are discussed above. The ocular manifestation of acute pandyautonomia have been described (with NEI).

Some patients with spinocerebellar degeneration have slow eye movements. We reported (with NEI) that they made abnormally slow refixational eye movements by the saccadic system (i.e., they were slow saccades) rather than by the voluntary pursuit system. That led to the proposal of a new conceptual scheme of how both normal and defective saccadic eye movements might be generated. Thus, quantitative recording of eye movements in a group of related patients with familial late-onset cerebellar ataxia has revealed new information about non-visual control of eye position, since the striking abnormality was a defective smooth-pursuit and fixation system. The patients showed evidence of various non-visual mechanisms of maintaining eye position that have not been previously delineated. Rebound nystagmus was shown to occur in normal individuals if fixation is eliminated -- however it becomes clinically apparent in patients with spinocerebellar degeneration because of a coexisting defect of visually-mediated fixation mechanisms. Accordingly, rebound nystagmus can be interpreted as a manifestation of one of the brain's compensatory mechanisms for maintaining eye position when visual systems are ineffective. The findings supported the suggestion that the cerebellum specifically: (a) helps maintain eccentric gaze; (b) produces smooth-pursuit eye movement; (c) modulates the amplitude of saccadic eye movements.

EM-histochemistry for calcium (with NEI), now reported, has shown its discrete localization within the sacs of the outer segment of the rod cells of the retina, suggesting that the sacs may have a function of calcium storage-and-discharge with photoexcitation analogous to that of the sarcoplasmic reticulum of muscle. The finding has importance in the hypotheses of photo-electrical coupling mechanisms.

Progressive blindness was reported in two children with acute lymphocytic leukemia intensively treated with chemotherapy and radiation and considered a possible complication of that therapy (with NEI).

Other: We have formally called attention to the possible menstrual hemorrhagic effect of aspirin incorporated into virtually every over-the-counter anti-dysmenorrheic preparation, on the basis of aspirin's known ability to reduce-clotting/increase-bleeding.

Significance to Bio-Medical Research and the Program of the Institute: These findings provide new information on the pathologic and pathogenic aspects of the various lower motor neuron disorders, peripheral neuropathies, and spinocerebellar degenerations, on the treatment of some, on animal-models of some of these disorders.

Proposed Course of Project: To more fully develop the interlinked basic and clinical studies underway directed toward clarification of the pathogenesis and identification of the eitology, and toward elaboration of means of treatment and prevention of these disorders.

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July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Myopathies

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To more fully elaborate the clinical, cultural, histochemical, biochemical, ultrastructural, electrophysiologic and immunologic abnormalities of patients with the various myopathies and certain other neuromuscular disorders. To further sub-classify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including tissue culture and ones listed above, applied to the patient's body fluids and tissues, especially to the muscle biopsy specimens. To treat myopathic disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

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Objectives To more fully elaborate the clinical, cultural, histochemical, biochemical, ultrastructural, electrophysiologic and immunologic abnormalities of patients with the various myopathies and certain other neuromuscular disorders. To further sub-classify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including tissue culture and the ones listed above, applied to the patient's body fluids and tissues, especially to the muscle biopsy specimens. To treat myopathic disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: A variety of techniques encompassing tissue culture, histochemistry, biochemistry, autoradiography, radionuclide scanning, electrophysiology, electronmicroscopy, and immunology are applied to patients with the various myopathies and induced animal-models thereof.

Patient Material: Patients and diagnostic material from Medical Neurology Branch patients and from outside patients from whom diagnostic muscle biopsies were obtained and sent here for study.

Major Findings:

Myopathies are non-neurogenic, primary or secondary diseases of muscle. Some, such as the dermatomyositis/polymyositis group, are often at least partially treatable but their cause and details of their probably "dysimmune" pathogenesis are not known; others are not treatable but their cause is known, e.g., genetic deficiencies of phosphorylase, phosphofructokinase, acid-maltase or carnitine-palmatyl-transferase; while still others, such as Duchenne muscular dystrophy and others bearing the name "dystrophy", are of unknown pathogenesis and are untreatable.

The polymyositis/dermatomyositis (PM/DM) disease-complex is an acquired disorder causing progressive deterioration of muscle in children and adults. The primary cause is not known but the pathogenic mechanism is considered "autoimmune" or "dysimmune". All patients were seriously incapacitated and many died before the introduction of anti-dysimmune therapy. Our studies involved improving diagnositc methods, seeking the cause, analyzing the pathogenic mechanisms, and improving methods of treatment.

The special variation of anti-dysimmune treatment we introduced to this disease 8 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) has continued to be, in our series (about 70 cases) and others, the single best available treatment for children and adults (without or with an associated cancer). It has the greatest benefit/side-effect ratio and is easiest to manage. However, because not all patients respond and because the side-effects of LT-HSDAD-Pred can be significant, we are defining some of the parameters of their immunologic response to prednisone and seeking predictive indicators thereof.

By use of T- and B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have established that in PM/DM patients, while HSDAD-Pred is clinically cumulatively effective for months and longer, its measurable effect on the peripheral circulating lymphocytes, using currently available techniques, lasts less tha 24 hrs; and these effects are more profound on the T-lymphocytes. We are now correlating these data with concurrently obtained blood levels of prednisone and prednisolone to determine any hypoabsorption or hypercatabolism of the drug (with P,CC). In patients, children and adults inadequately responding to HSDAD-Pred we have found previously on an occasional-case basis some remarkable therapeutic responses to added azathiaprine (3 mg/kg/d) -- to more clearly establish the efficacy of azathiaprine (plus an unchanged dose of prednisone), a double-blind trial is being conducted in otherwise refractory DM/PM patients, 9 to date. We are also measuring the patients' lymphocyte responses to azathiaprine.

Pulmonary interstitial fibrosis we have found to be common in our DM/PM patients -- detailed pulmonary functions are now being evaluated in all our DM/PM patients, 11 to date (with NHLI). Cardiac involvement (conduction blocks, arrhythmias, systolic mitral prolapse) was demonstrated in the majority of 20 cases of DM/PM studied with non-invasive cardiologic techniques with NHLI, V. A. Hosp. DC, and Georgetown Univ.).

DM/PM is presumably a dysimmune response to an exogenous antigen, specific treatment of which could be curative (as opposed to the merely suppressive action of all current treatments). We are now seeking that antigen, and some preliminary clues have been found.

Massive subcutaneous calcification can be a crippling complication of childhood DM. We have found a new way to clinically detect it in its early stages, isotopically with ^{99m}Tc-diphosphonate body scanning (with NM,CC) (for new microscopic detection methods, v.i.). Our therapeutic trials with diphosphonate have not been beneficial (also not beneficial in a patient with myositis ossificans). However, in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathiaprine-HSDAD-Pred program. The reasons for the calcification are being sought by our histochemical, electronmiscroscopic, and autoradiographic studies (v.i.).

The exact mechanism of muscle damage in PM/DM is unknown. Previously we have reported immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, findings supporting our earlier hypothesis that an aspect of the muscle damage may be vascular. While disorders of both immunoglobulins (produced by B-lymphocytes) and direct immunocyte toxicity (T-lymphocytes) might be occurring in all cases of PM/DM, perhaps the former (deposited as intravascular immune complexes) are more muscle-damaging in the childhood form and latter are more pathogenic in the adult form. We are now attempting to correlate a possible vascular mechanism with our histochemical finding of preferential perifascicular involvement (occurring especially in childhood DM), as well as relating our findings in patients to our experimental animal model of muscle ischemia (v.i.). It is conceivable that the immunologic abnormalities of PM/DM are ultimately provoked by a viral infection; however, our attempts to see or "rescue" a virus from PM/DM patients' muscle directly after tissue culture of it, or after animal inoculation, thus far have been negative with ID-NINCDS and NCI); nor has reverse transcriptase been found so far.

A new and possible disease-characteristic histochemical finding in PM/DM patients of microscopic foci calcium accumulation in collagenous connective tissue of muscle and subcutaneous regions, demonstrated histochemically at both the light-microscopic (alizarin-red method) and EM (pyroantimonate method) levels is being published. Its pathogenesis is being sought, as is its correlation with our positive ^{99m}Tc-diphosphonate patient-scans for calcium, our autoradiographs of patient skin and muscle biopsies for calcium, and our previously described highly-disease-characteristic histochemical finding of alkaline-phosphatase staining in the intramuscular connective tissue (also being published).

Duchenne muscular dystrophy (DMD) is an hereditary progressive deterioration of muscle in children, usually causing wheelchair or bed confinement by age 12 and death by age 20 years. Cause and treatment are not known. Our studies involve improving diagnostic methods, seeking the basic cause, and trying experimental therapy when available. We also seek fuller understanding of the basic biology and pathologic responses of muscle. The other myopathies and the other neuromuscular diseases of unknown nature which we study are often devastating to children or adults, and in studying them our general objectives are the same.

Current competing hypotheses of the pathogenesis of DMD are: (a) primary or secondary defect of blood supply to muscle, (b) primary defect of energy source within the muscle fiber, and (c) primary muscle-fiber plasmalemmal defect. Although many others favor c, we favor a or b. Some of the findings in DMD muscle considered by others to be supportive of c we consider to be invalid results or not distinguishing between c and b. For example, the defect of erythrocyte morphology reported by others we have reported as not present in our controlled studies. A further aspect of erythrocyte normality is our finding that in 8 DMD patients, ^{51}chromium-labeled erythrocyte survival was normal (with H,CC). The defect of the muscle-fiber plasmalemma evident by peroxidase penetration reported by others we are reporting not to be disease-specific. The alteration of muscle homogenate adenylcyclase reported by others we have reservations about because (i) it was not exclusive to DMD, and (ii) its cellular site (presumed by them to be muscle-fiber plasmalemma) could not be determined from those biochemical studies. Our own newly developed histochemical methods for adenycyclase (AC), utilizing AMP-PNP as the substrate and Ca^{++} (which doesn't inhibit AC like Pb^{++} does) as the capture agent, shows highest muscle AC in blood vessels and almost none detectable in normal muscle at shorter incubation times; but in regenerative fiber fairly high amounts of AC are evident diffusely throughout the T-tubule (± SR) network and plasmalemma. This technique we also developed at the EM-histochemical level and showed AC precisely localized to the plasmalemmal and T-tubule membranes. In DMD biopsies, many fibers, often appearing normal with other histochemical reactions, show subtle regenerative features with the AC histochemical technique.

In DMD the plasmalemma has long been known to be leaky, evidenced by elevated CPK and other "muscle enzymes" in the serum, but that certainly does not specify a primary plasmalemmal defect (v.i.). Human regenerative fibers have certain fetal isozymes -- e.g., LDH-1,2 has been known and we now confirm the recent finding of CPK-MB. We have introduced the use of elevations of CPK-MB isozyme in serum (and in muscle biopsies) for detecting DMD carriers. Our other new method of carrier detection is elevated serum hemopexin (v.i.). These together we use as fine-tuning for DMD-carrier detection, identifying carriers missed by all other carrier-detection methods. Our data are also being reported in the context that elevated plasma CPK-MB is not diagnostic of myocardial infarction as some cardiologists have reported -- we find it elevated in any kind of leaky skeletal-muscle disease (e.g., DMD and DM/PM). Our new test of ^{99m}Tc-disphosphonate forearm scanning 24 hrs after ischemic exercise (6 hrs after isotope injection) did not detect DMD carriers (or patients) like it did phosphorylase and phosphofructokinase deficiency patients, (with NM, CC).

Hemopexin, an inducible, liver-produced, heme-transport protein, was first discovered elevated in DMD patients and carriers by Askanas a few years ago. We have confirmed that, and supported the hypothesis that the elevation is due to subtle myoglobin leakage from minimally damaged muscle fibers by finding: (a) with a sensitive radioimmunoassay, myoglobin leakage into the serum of nearly all DMD patients and half of the carriers (with Columbia); (b) hemopexin elevation in monkeys with experimentally crushed muscle or injected with myoglobin, which persisted long after CPK levels returned to normal (in the crush studies). A large number of neuromuscular-disease patients were surveyed with the immunodiffusion assay for hemopexin quantitation. Only active myopathies, especially dermatomyositis/polymyositis and DMD patients and carriers, and myasthenia gravis patients, had hemopexin elevations (since myasthenia gravis patients have no evidence of muscle-cell leakage the possibility of their hemopexin elevation being a very special dysimmune phenomena is being investigated). We are beginning to study hemopexin molecular turnover in a variety of neuromuscular-disease patients.

Our new model involving the blockage of leg-muscle anaerobic glycolysis by intra-aortic iodoacetate, which results in the secondary loss of plasmalemmal membrane integrity of the damaged fibers (v.i.), establishes an experimental basis for at least the possibility of hypothesis b re. DMD pathogenesis (above), in harmony with our clinical demonstration that in muscle phosphorylase deficiency ischemic exercise precipitates acute breakdown of the plasmalemmal barrier, allowing marked creatine-phosphokinase (CPK) egress and calcium ingress (detected by scanning with ^{99m}Tc-diphosphonate).

Our ischemia hypothesis for DMD, which proposes a functional defect on the arterial side of the vascular tree, has been based on our studies of the histochemopathology of DMD muscle and of our experimental ischemic myopathy in animals. As yet, an ischemia mechanism, although possible in DMD patients, has not been demonstrated in them directly (that would be the next logical step). However, our study in press demonstrates that in known ischemic human muscle, caused by occlusive vascular disease, the histochemical pattern of the regions of lesser involvement of muscle (groups of necrotic or regenerating fibers in fields of normality) is indistinguishable from that of early DMD and of our experimental ischemic myopathy in animals, thus being at least harmonious with the ischemia hypothesis (with VAH, D.C.). To date, none of the studies attempting to detract from our ischemia hypothesis is conclusive, nor even incisive.

A new method to measure capillary blood flow, utilizing the Doppler effect, has been developed by others. When another machine becomes available we will measure muscle blood flow directly during our diagnostic open muscle biopsy, in DMD, DM/PM and other neuromuscular diseases in which ischemia mechanisms have been considered. Platelet uptake of serotonin, which 10 yrs ago we found impaired in DMD, is being re-measured with a new technique.

Although phosphodiesterase (PDE) inhibition is considered to reduce peripheral arteriolar resistance and increase peripheral blood-flow, a PDE-inhibitor has not benefited our DMD patients; nor has allopurinol.

Using the ischemia-myopathy model to study details of the mechanisms of muscle fiber damage, we have reported the development of a new, rapid and simple radioisotopic method, based on uptake of ^{99m}Tc-diphosphonate, of quantitating active skeletal muscle damage in experimental animals -- that uptake was directly correlated with other parameters of muscle damage, loss of muscle K$^+$ and elevation of plasma CPK, and also directly correlated with uptake of ^{3}H-diphosphonate and with atomic-absorption-measured calcium uptake, that calcium accumulation presumably the basis for the diphosphonate localization. Our on-going autoradiography shows the uptake of ^{3}H-diphosphonate to be within muscle fibers, ones necrotic and even intact ones (presumably prenecrotic or perhaps reversibly injured) -- this correlates well (in histochemically-stained serial sections) with calcium accumulation within, and complete loss of phosphorylase/glycogen staining from, those fibers. In press is our report showing that calcium accumulation in damaged muscle fibers is also evident by light-and electronmicroscopy-histochemistry in various human disorders -- it is not disease-specific but it is a new way to identify damaged fibers, including minimally affected ones not identifiable by other methods. Our electronmicroscopy-histochemical study, using pyroantimonate, also in press, shows the calcium increase in non-specifically damaged fibers to be in mitochondria>sarcoplasmic reticulum>myofibrils. Nuclei (nucleoli and heterochromatin) are also calciumed. This pattern of organellar calciuming is the same one we find when normal intact muscle is soaked in a high-calcium solution.

From all of these studies we have formulated a new hypothesis covering molecular aspects of muscle fiber damage, as follows: (a) any damage to the plasmalemma -- be it, as we have demonstrated, exogenous (e.g., trauma, ischemia), endogenous deficiency of ATP-generating energy-supply (e.g., phosphorylase, phosphofructokinase, and carnitine-palmatyl-transferase deficiencies, experimental poisoning of glyceraldehyde-3-phosphate dehydrogenase with iodoacetate) or, conceivable but not tested, a direct primary or secondary plasmalemmal defect -- can break the normal plasmalemmal barrier to calcium

(Ca_o^{++} / Ca_i^{++} is normally about 10,000-20,000/1); (b) the influxing Ca^{++} is taken up, as we demonstrated, by those organelles known to normally have the greatest capacity to do so, i.e., mitochondria>SR>myofibrils, and nuclei; (c) this heavy calcium accumulation eventually can damage organellar function -- e.g., a high Ca^{++} is known to reduce mitochondrial ATP synthesis, which presumably would lead to further impairment of the plasmalemmal barrier and increasing damage of the muscle fiber, and it also causes myofibrillar contracture and activates proteolytic processes, and may affect DNA and RNA; (d) thus, excess calcium within muscle (or other) cells may be the "ultimate molecular assassin", pushing damaged muscle fibers past their point of no return; (e) in contrast to the lethal cascade of events proposed to result from unrestricted or marked calcium ingress, if the increase of intracellular calcium is only slight and restricted, it might be relatively benign, and might actually be the spark for repair/regeneration or hypertrophy of muscle fibers, perhaps by displacing histones or other suppressor proteins to activate (derepress) DNA-RNA-transcription and hence RNA-protein translation systems in cells destined to survive, and it might also be the spark for cellular proliferation through activation (derepression) of DNA-replicating systems in minimally injured resting satellite cells, the cells destined to repopulate the tissue with myoblasts for reconstitution of muscle fibers.

Our theoretical and experimental data prompted our finding that computer-analyzed patient-scanning with ^{99m}Tc-diphosphonate can be used to detect abnormal uptake, presumably based on abnormal accumulations of calcium, in various disorders of muscle (with NM, CC). There is: (a) greatest uptake in the polymyositis/dermatomyositis complex (probably related to damaged muscle fibers and enhanced because of diffuse radiologically-invisible but histochemically and electromicroscopically-evident calcium in connective tissue of muscle and subcutaneous regions), in the presence of high, moderate or normal serum CPK; (b) moderate uptake in DMD patients, who have constantly very high serum CPK; (c) normal or minimally increased uptake in neurogenic involvement; (d) very high uptake in phosphorylase-deficiency and phosphofructokinase-deficiency muscle acutely injured by ischemic exercise and in muscle acutely injured by trauma (viz., at the site of muscle biopsy).

We are reporting that the histochemically "dark" muscle fibers characteristic of DMD are artifactually contracted and contain excess calcium, probably having incurred plasmalemmal injury during biopsy because of their abnormal fragility; such fragility we suggest is characteristic of DMD fibers. There are also more non-dark fibers calciumed in DMD than other myopathies, probably representing fragile fibers developing plasmalemmal injury in vivo as a reflection of the basic disease process. Other muscle-fiber markers of plasmalemmal injury we are studying are accumulated normally-barriered extracellular proteins, e.g., hemopexin, immunoglobulins and albumin.

A detailed study of mechanisms of muscle-cell injury in relation to Duchenne dystrophy is in press, or is a survey chapter (>500 references) of the biology of the muscle cell in relation to myopathies.

Other myopathies. Tissue culture of human muscle biopsies provides living muscle fibers growing free of all neural, vascular, and humoral factors present in the patients. After we developed a new program (reported two years ago) for achieving abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, and for precisely selecting certain fibers for enzyme-histochemistry, immunohistochemistry, EM, or EM-histochemistry, we have turned our attention to culturing the biochemically and the morphologically distinct myopathies.

In adult-onset acid maltase deficiency we earlier reported in one patient the first "reincarnation" in cultured muscle fibers of the biochemical and morphological defects characteristic of the disease, i.e., the first proof of endogenous myopathos of a muscular dystrophy, -- the muscle fibers in the biopsy and the ones newly grown in culture were identical by histology (vacuoles), histochemistry (acid phosphatase high in the vacuolar and pre-vacuolar regions), electronmicroscopy (glycogen accumulated in lysosomes) and biochemistry (with Columbia) (absent acid maltase by the natural-substrate method, 5-10% of normal acid maltase by the artifical substrate method, normal neutral maltase), normal kinetics of neutral and the trace-amount acid maltase, elevated acid phosphatase. That provided a new test system for manipulations directed toward the treatment or prevention of muscle-fiber damage in this disease without inducing any risk to the patient. Now we have shown similar reincarnation of the severe biochemical and morophologic defects in 4 additional chronic-infantile and 3 adult cases of acid-maltase deficiency, and partial defects in 3 heterozygotes. In the patients, a medium-chain-triglyceride ketogenic diet produced some subjective improvement -- this will be further evaluated.

We recently reincarnated a second metabolically distinct myopathy, phosphofructokinase (PFK) deficiency. Extremely reduced levels of that enzyme were found in muscle fibers cultured from a patient with a new syndrome of combined muscle and erythrocyte PFK deficiency and hypolipoproteinemia (with CP-CC and Columbia). Growth of that muscle in culture was enhanced by β-hydroxybutyrate added to the medium, a ketone-body providing one of the alternate energy sources which that muscle must be depending on since its glucose-utilization pathway is defective. In the patient, forearm ischemic exercise was shown to produce electrically-silent contracture, preferential damage to type-II muscle fibers (calcium accumulation when mild and frank necrosis when severe) and positive ^{99m}Tc-diphosphonate scan.

In phosphorylase deficiency, a third (actually our first tissue-culture) glycogenolytic-enzyme defect of muscle, we have in 3 additional cases confirmed our previous finding that the enzymatic absence from the muscle fibers is "cured" with their growth in culture (i.e., a remarkable and unexpected recovery of phosphorylase activity is seen in fibers in the regenerative state in culture and in vivo. We have also now shown that phosphorylase is normal muscle phosphorylase (not a genetically different type) by histochemical staining of isozyme elctrofocused gels, thereby demonstrating a true rejuvenation of an enzyme genetically programmed ultimately to be deficient in mature fibers (with Hopital des Enfants Malades, Paris). The therapeutic thrust needed now is to provoke maintenance of that phosphorylase in the mature fibers of the patient.

In two phosphorylase-deficiency patients, utilizing customized treadmill exercise to achieve reproducible serum CPK rises and muscle cramps under standard conditions, we found that i.v. triglycerides prevented the CPK rise but not the cramps whereas glucose-insulin prevented both. In one patient, an oral medium-chain-triglyceride ketogenic diet for two weeks also prevented the exercise-induced CPK rise. Thus acute-intravenous and chronic-oral triglyceride therapy can prevent exercise-induced rhabdomyolysis, reflecting plasmalemmal breakdown from failure of ATP supply (since it did not mitigate the cramps as did glucose-insulin, different mechanisms must be involved).

Because we have previously shown in phosphorylase-deficiency patients the presence of a small amount of phosphorylase in regenerating fibers and because phosphorylase is activitable via cAMP, we are seeking a beneficial effect of a phosphodiesterase-inhibitor.

A new diagnostic test has been introduced and found positive in muscle phosphorylase deficiency (many other neuromuscular disorders are negative): ^{99m}Tc-diphosphonate scanning shows increase of calcium in forearm muscle 24 hrs after the standard diagnostic forearm ischemic-exercise test (FIET), and this uptake approximately parallels serum CPK elevations. Correlated histochemical and our new autoradiographic technique for localizing the clinically-administered gamma-emitting ^{99m}Tc-disphosphonate documented uptake of the tracer into injured, calciumed muscle fibers, and they showed preferential injury of type-II muscle fibers after FIET in muscle-phosphorylase deficiency. (This is part of our effort to develop techniques for doing autoradiography of patient biopsy samples following injection of some of the short-lived gamma-emitting radionuclides used for patient-scanning, to establish direct scanning-histoautoradiographic correlations.)

Single-fiber EMG of the patients during and after FIET established electrical failure of all motor units during FIET, and later no abnormal jitter and no motor-unit densification (i.e., no foreign reinnervation of type-I muscle fibers) -- thus although both fiber types fail during FIET, the I-fibers were not remarkably damaged (perhaps because of greater supplies of, and ability to use, endogenous non-glycolytic substrates and myoglobin-associated O_2, or could their axons have "protectively" shut off. These findings provide a basis for formulating alternate-pathway-substrate therapy in MPD.

A new model of defects in muscle glycogenolysis/glycolysis was produced using iodoacetate (which inhibits glyceraldehyde-3-phosphate dehydrogenase) intra-aortically plus repetitive sciatic-nerve stimulation: there was an electrically-silent contracture and 24 hr later a positive ^{99m}Tc-disphosphonate scintiscan, and low-oxidative type-II muscle fibers were preferentially injured and with histochemically evident calcium-uptake early and later frank necrosis. This model makes possible controlled analyses of the stages of intrinsic energy-depletion muscle injury, and subsequent repair, as well as evaluation of various alternate-pathway therapies applicable to human GG/Gly defects, e.g., phosphorylase or phosphofructokinase deficiency.

A new syndrome of fasting-induced (presumably) fatal rhabdomyolysis has been found. The biochemical cause is unknown -- none of the known biochemical defects of muscle is present and anaerobic glycolysis is intact. Lipid droplets were slightly increased in muscle fibers and biochemically carnitine palmatyl transferase was 2X normal -- an ATP-generating lipid-metabolism-defect is suspected (with Columbia).

Because carnitine is essential for the transport of long-chain fatty-acids into muscle mitochondria for oxidation and because carnitine deficiency causes a progressive myopathy, we are now studying autoradiographically the time-course uptake and localization of ^{3}H-carnitine into specific muscle-fiber types of animals and of tissue-cultured muscle. We will also develop a method for the same studies of patient muscle-biopsies in vitro.

A morphologically characteristic defect, "cabbage bodies" (multilamilated material in lysosomes) in muscle fibers of a patient with a chronic myopathy, were reproduced in his cultured muscle fibers. Although the biochemical defect is not known, both the biopsied and cultured muscle had elevated acid phosphatase, suggesting the patient has a defect of a yet-unpinpointed lysosomal hydrolytic enzyme (with Hopital des Enfants Malades, Paris). In that and our other cultures of human and animal muscle we are assaying 12 different lysosomal enzymes to search for defects thereof (with Hopital des Enfants Malades, Paris).

"Ragged-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical manifestation in limb muscles of the heterogeneous syndrome of oculocraniosomatic neuromuscular disorder with ragged-red fibers (OCSNMD-RR) (the patients often having lacticacidosis and often ophthalmoplegia. Some patients have a syndrome of small stature, seizures, mental impairment, and lacticacidosis. In limb muscle cultured from such patients, most of the mitochondrial changes, including increased number and greatly increased size of mitochondria, wide distorted "twisted-ribbon" cristae, and "mushy" inclusion material, have been re-incarnated, although the mitochondrial crystal-like inclusions have not yet been (perhaps they take longer to develop). The same changes were produced in normal human muscle cultures after 2 days exposure to dinitrophenol, and the ragged-red-fiber cultured muscle was extremely susceptible to worsening of the in vitro changes by dinitrophenol. This demonstrates a mitochondrial defect which is reproducible in cultured muscle fibers and provides a test-system for seeking a possible genetic or occult-infectious basis. In the original biopsies and in the cultures the mushy and crystalline inclusions lacked cytochrome oxidase staining by our EM-cytochemistry.

Clinically, some OCSNMD-RR patients with ophthalmoplegia have cerebellar ataxia, mental impairment, some denervation evident in muscle biopsy, and spinal-fluid protein increase -- of such patients, some have by CAT-scan a small brain-stem (shown by enlarged 4th ventricle and pre-pontine cisterns) and a decreased attenuation coefficient of cerebral white-matter, changes absolutely correlated with electrophysiologically-determined delay of the bilateral late-response of the blink reflex (with SNB and EEG).

Soft-tissue scanning of several OCSNMD-RR patients with ^{99m}Tc-diphosphonate has been positive, and curious in view of their normal serum CPKs. By pyroantimonate EM-cytochemistry calcium was not increased in the abnormal mitochondria of ragged-red fibers.

In muscle cultured from a Dutch patient (with Wilhelmina Gasthuis) with X-linked recessive severe-infantile muscle-fiber-hypotrophy-and-central nuclei, there was very abnormal proliferation with bizarre nuclei -- so much so that from a cloned single myoblast a cell line was started and carried through 6 passages, until it was lost because of incubator failure. Biopsies from the carriers will be used for a repeat attempt to develop a human muscle-cell line.

Abundant leptomeres were found in muscle cultured from 5 patients with acid-maltase deficiency and 1 with autophagic vacuolar myopathy but not in our other cultured normal or pathologic muscle. Similar abundant leptomeres were reproduced in normal human muscle fiber cultures exposed to dinitrophenol.

In tissue cultures of "normal" chick embryo skeletal muscle we were able to induce certain bizarre changes of mitochondrial morphology by pulses of dinitrophenol (DNP), but did not produce all the mitochondrial changes occurring in the human "ragged-red" fibers. In the course of that study we found, and reported, that DNP markedly promoted the detectability of avian oncornavirus in all such "normal" chick-muscle cultures -- virus was evident morphologically (C-particles) and by complement-fixation titers (COFAL). That demonstrated that the "normal" chick embryo muscle cultures so frequently used by various investigators for physiologic, biochemical, immunologic and developmental studies are actually virally-contaminated test objects. The question of whether that oncornavirus has a "normal" role in development of "normal" chick muscle in vivo or in vitro was raised by that study. Our finding the C-particles exclusively in dilations attributed to T-tubules raises the intriguing possibility that T-tubules might be the aqueducts of virus infestation of or shedding from muscle fibers. The question of viruses harbored in mitochondria (as "mitochondriophages", analogous to bacteriophages) is raised by the C-particles being provoked by DNP, an uncoupler of mitochondria-oxidative phosphorylation. Finally, we have raised the speculation that such "mitochondriophages" might be responsible for the mitochondrial changes in the ragged-red-fiber diseases.

Pyruvate kinase assayed in cultured human muscle by electrofocusing and histochemical staining was shown for the first time to have fetal isozymes (with Hopital des Enfants Malades, Paris).

In biopsies of 5 patients with sporadic chronic myopathies we have by electronmicroscopy found under the plasmalemma long strands suggesting viral material. Its nature and significance are being studied by cytochemical stains for DNA and RNA at the ultrastructural level, assays of reverse transcriptase, and attempts to reproduce it in culture. Our series of 12 patients with idiopathic vacuolar myopathy, some reincarnated in culture, is being reviewed in detail for publication (with Wilhelmina Gasthuis).

Neuromuscular disorders of uncertain classification. We continue to study the selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype IIB fibers, which we have shown to be the basis of cachectic atrophy accompanying cancer and other chronic debilitating disorders. Evaluation of the cause of type-II fiber atrophy in cancer patients, theoretical mechanisms of which we published last year, is important because this "remote-effect" muscle weakness is often the most crippling aspect of cancer -- if the molecular mechanism can be discovered it might be treatable independently of treatment and response of the cancer itself. And improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy.

Malignant hyperthermia-rigidity (MHR) is a syndrome, 70% fatal, of acute rise of body temperature and muscle rigidity during general anaesthesia. A number of the patients have underlying not-well-defined neuromuscular disorders. In one MHR patient, and his father, we are reporting central-core disease (CCD), with its typical type-I muscle-fiber predominance (with Children's Hospital, D.C.). Because we have another family with CCD and MHR and there are in the literature two other such families, we have issued a caution to all our CCD patients and their physicians regarding the possibility of MHR during general anaesthesia. The mechanism(s) of the attack of MHR is not known. It appears that there is an excess of free intracellular calcium in the muscle fiber, which we propose might be due to an effect of the anaesthetic or muscle-relaxant agent on the calcium-barrier function of the plasmalemma (v.s.) (rather than the SR as proposed by others). We are now investigating why central core disease, which we have earlier postulated to be due to a pre-natal monophasic neuropathy mainly affecting the type-II units, should predispose to the development of MHR. We are also working on new tests for specific clinical and muscle-biopsy diagnostic tests to identify patients predisposed to develop MHR.

Cross category and basic aspects. None of the eye muscle fiber-types is histochemically exactly like a limb-muscle fiber-type. Being published is our demonstration of their normal histochemical patterns in Rhesus monkey and identification of 3 types, "fine", "granular" and "coarse". The first two have one endplate per fiber and probably are different types of twitch fibers; the last has multiple endplates and may be a tonic fiber. Following denervation the first two developed diffuse extrajunctional acetylcholine receptors but the coarse fibers did not -- no fibers were positive beyond 13 weeks post-denervation. To secure more adequate samples with less discomfort a new needle-biopsy device has been designed. (Needle muscle biopsies are satisfactory when only a small amount of tissue is required for non-histochemical purposes, e.g., for tissue-culture or biochemistry.)

Mammalian plasmalemmal membranes have received most of our biochemical attention, through use of a battery of techniques. Results of the following studies are now being published or have been presented at meetings. Pure fractions of rat plasmalemma were obtained, and the methods perfected so that adequate quantities of plasmalemmal-membrane can from normal human muscle be obtained from limb amputation or radical mastectomy, and even from pathologic muscle biopsies for limited studies. With the plasmalemmal fractions and subfractions, methods have been established for studying acetylcholine receptor, acetylcholinesterase, Na$^+$-K$^+$ ATPase (Na$^+$-stimulated phosphorylation), divalent-cation (viz., Ca^{++}) binding/transport, Ca^{++}-stimulated ATPase, adenylcyclase, guanylate cyclase and catecholamine receptors. Some of these have been studied in sarcoplasmic reticulum (SR) fractions as well. Elucidated have been detailed properties of: (a) the adenylcyclase (i.e., fractional localization, kinetics, catecholamine-activation [the plasmalemmal AC was demonstrated to be a β_2 adrenergic receptor], guanylyl-inidodiphosphate activation, insulin and glucagon inhibition, all in animal "red" cf. "white" muscle and also the responses to denervation in "red" cf. "white" muscle; (b) differences between the catecholamine-sensitive adenylcyclase and β-receptor-binding (by use of ^{3}H-dihydroalprenol); (c) in human muscle the catecholamine-sensitive and GTP-stimulated adenylcyclase is predominately localized in the plasmalemmal fraction; (d) the human plasmalemmal adenylcylcase, on the basis of various agonist and blocker effects, showing that it is closely associated with β_2-adrenergic receptors, that its catecholamine-responsivity (but not its basal activity) is enhanced by GTP (and to a lesser extent by ITP), and that GMP-PNP stimulated both the β-catacholamine-responsive and basal adenylcyclase, and that both GTP and GMP-PNP act at the same site, indicating guanyl nucleotides are potent regulators of this plasmalemmal enzyme; (e) adenylcyclase (AC) in regenerating animal muscle, indicating that 5 days after crush the basal and isoproterenol-stimulated AC was decreased 30% in the plasmalemmal fraction and enhanced 40-60% in the SR and mitochondrial fractions -- since ^{45}Ca-uptake by the plasmalemma was increased 60% (and unaffected in the SR fraction) and Ca^{++} can inhibit AC, it is possible that the decreased AC in the plasmalemma in the in vitro assay was due to calcium accumulation; (f) guanylate cyclase (GC) re. subcellular distribution (highest in plasmalemma), kinetic properties, ionic requirements, molecular weight (about 300,000 for the plasmalemmal GC and 90,000 for the soluble form), and response to denervation (2-fold increase by 2 weeks of plasmalemmal and soluble GC, with unchanged kinetic and chromatographic properties, accompanied by 50% increase of cGMP-PDE activity; (g) the plasmalemmal protein phosphorylation (i.e., Na$^+$ enhancement blocked by K$^+$, phosphorprotein state suggesting an acylphosphate bond, high turnover-rate suggesting it is a functional intermediate of Na$^+$K$^+$ ATPase, and molecular weight); (h) the effect of denervation on this transport-ATPase system, (i) the Ca^{++} uptake and release by human SR (i.e., by use of specific antibodies made against SR which block the Ca^{++} uptake and inhibit adenylcyclase but do not affect ATPase activity, suggesting different localization of these functions within the SR; (j) the role of bound-calcium in the regulation of Ca^{++} transport by SR; and (k) physicochemical properties of ACh-receptor cf. acetylcholinesterase. These assays are now being used to elucidate biochemical defects of plasmalemmal or SR function in muscle biopsies from patients with various neuromuscular disorders (see our Episodic-Weakness-and-Myotonia project).

Insulin-receptor-function analysis of our cultured chick muscle fibers, intended as the first step toward studies of diabetic neuropathy patients (with Georgetown U.), showed that the uninnervated embryoid cultured fibers had no functional insulin receptors, in contrast to mature innervated fibers *in vivo*.

We have established microtechniques for cAMP and cGMP phosphodiesterases (PDEs), and are applying them to a portion of our patient muscle biopsies, and patient platelets. We have also applied it to cultured chick muscle and demonstrated a strong in vitro inhibitory effect on cAMP-PDE of phthalazinol. We are also studying the effect of dibutyryl-cAMP and phthalazinol on growth and maturation of human and animal muscle fibers in culture.

Acetylcholinesterase (AChE) subcellular distribution and properties showed: (a) majority present extrajunctionally, mostly in microsomes-SR but some in the soluble phase; (b) after denervation, the early AChE reduction is mainly in the microsomal-SR fractions; (c) the 16-S form is restricted to the neuromuscular junction (per other), while the microsomal-SR fraction had mainly 4-S (and this was the AChE abruptly reduced after denervation) and the plasmalemma had only 10-S and 16-S. Synthesis, degradation and release of the different molecular forms of AChE will now be studied in normal and various pathologic circumstances.

Significance to Bio-Medical Research and the Program of the Institute: These findings provide new information on the pathologic and pathogenic aspects of the various myopathies, on the treatment of some, and on animal-models of some of the myopathies.

Proposed Course of Project: The studies underway are part of a long-term project consisting of interrelated studies which will continue for several years.

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Myasthenia Gravis (MG)

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SECTION

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS: 4.8	PROFESSIONAL: 3.5	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To apply clinical, immunologic, histochemical, pharmacologic, electrophysiologic, autoradiographic, radionuclide-scanning, tissue-culture and electronmicroscopic techniques to investigate the etiology and pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Names, laboratory and Institute Affiliations:

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Cooperating Units:

University of Maryland, School of Medicine
Neuro-Immunology Branch, NINCDS

Project Description:

Objectives: To apply clinical, immunologic, histochemical, pharmacologic, electrophysiologic, autoradiographic, radionuclide-scanning, tissue-culture and electronmicroscopic techniques to investigate the etiology and pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Methods Employed: A variety of basic and clinical investigative techniques, as detailed below, were applied to patients with myasthenia gravis and other disorders of neuromuscular transmission, and to include animal-models thereof.

Patient Material: Myasthenia gravis patients, and patients with other disorders of neuromuscular transmission, participated in the investigative studies and therapeutic trials. Sera, muscle, thymus and other tissue were obtained during diagnostic or therapeutic procedures from these Medical Neurology Branch patients.

Major Findings:

Myasthenia gravis (MG) is an acquired disorder affecting transmission at the neuromuscular junction, mainly in adults and older children. The primary cause is not known but the pathogenic mechanism is considered to be "autoimmune" or "dysimmune". Untreated patients usually are seriously handicapped and many die. Palliative treatment with anticholinesterases and antipathogenic treatment consisting of thymectomy, ACTH and, most recently, prednisone has helped considerably but much disability, some fatality, and drug side-effects still occur. Our studies involve improving diagnostic methods, seeking the cause and analyzing the pathogenic mechanisms, and improving methods of treatment. We also seek fuller understanding of the basic biologic and pathologic responses of the neuromuscular junction.

Two years ago we reported the first identification of a factor, an IgG, in the sera of MG patients which blocks binding of alpha-bungarotoxin (αBT) to the human junctional acetylcholine receptor (AChR) at the normal neuromuscular junction (41% of MG patients) and extrajunctional AChR of denervated human fibers (72% of MG patients, including all who had a thymoma), i.e., an "antireceptor antibody". We next reported that all MG patients having an IgG "antimuscle antibody" (first found by others) also had the blocking factor (although only half having the latter had the former) (the only discordant finding being in one non-myasthenic polymyositis patient with thymoma who had antimuscle antibody but no detectable blocking factor) and suggested that these may be the same antibody or, if different, virtually always co-produced. Our junctional localization of the blocking factor put it in the correct position to impair neuromuscular transmission and cause the weakness of MG. MG sera positive for blocking-factor were shown to block the binding of αBT to the diffuse extrajunctional receptors of aneurally cultured human, rat, and chick muscle fibers (additional studies of extrajunctional ACh receptors are detailed in our ALS Project).

On the basis of those studies and our EAMG studies (v.i.) we have now hypothesized the following: (a) two subsets of acetylcholine receptor (AChR), a "junctional" (J) and an "extrajunctional" (E) form; (b) both J and E AChR occur at the neuromuscular junction but extrajunctionally only E-AChR is present; (c) the IgG blocking factor in human MG is directed mainly against the E type of AChR while that of the EAMG model we worked with is mainly against the J-AChR; (d) the diffuse AChR of thymic epithelial cells, v.i., may be E-type.

From our work with the induced autoimmune model (rabbits injected with electric-fish AChR), originated by others, of experimental allergic MG (EAMG) (with U. Maryland) we have in-press our demonstration of: (a) binding of that rabbit sera to human neuromuscular junctions but not to extrajunctional receptor of denervated fibers at light-microscopic resolutions; that junctional binding of EAMG sera was not blocked by blocking-factor-positive (or negative) MG sera, only partially blocked by αBT, and not blocked by carbamylcholine, decamethonium or tubocurarine; (b) binding of it by electronmicroscopic resolution to the plasmalemma diffusely in rat and chick muscle fibers in tissue culture; (c) binding of that rabbit sera to the original antigen in a radioimmunoassay we developed (with IB), but no binding of blocking-factor-positive (or negative) MG sera to that antigen; (d) similarities but also distinct differences of the model with human MG, indicating it is not a perfect model of the latter although it could still be a model-in-principle. We are now exploring for blocking factor in another potential MG-model, the rhesus monkey with MG-like thymus hyperplasia induced by penicillamine.

Our electronmiscroscopic-immunohistochemistry demonstrated junctional αBT bindings presumably localizing AChR, at both the muscle post-synaptic plasmalemma and, to a lesser extent, at the axonal pre-synaptic plasmalemma, indicating that the IgG blocking factor, if indeed pathogenic, would act both post- and pre-synaptically in MG. Data from other types of studies by others support a pre- as well as a post-synaptic locus of AChR in normal neuromuscular junctions. But because the pre-synaptic localization is considered by others to be a diffusion artifact, current experiments are directed toward study αBT-binding to motor axonal terminals in isolation.

B-lymphocytes presumably are the cells making the junctionotoxic IgG in MG, but why? That the thymus is involved in many cases of MG is well known, but its role is not. In the thymus we have now demonstrated that the epithelial cells, typically hyperplastic in MG, all contain AChR demonstrable histochemically by αBT binding (with IB and Washington Univ.). This fulfills a step in our earlier hypothesis that the mechanism of MG might be an alteration of thymic epithelial cells e.g., by an exogenous virus, making them "foreign", in response to which B-cells make anti-AChR antibody (and perhaps T-lymphocytes also react) that co-reacts with junctional AChR to cause paralysis. Because the thymic epithelial cell is by others, considered pluripotential, this same hypothetical pathogenic mechanism could apply to other dysimmune diseases in respect to other thymic epithelial cell molecules altered to become antigen. Indeed, the multifactorial nature of the dysimmune phenomena in MG was re-emphasized by our report of anti-native-DNA antibody in the sera of a significant number of patients (with NIAMDD). We have also found serum hemopexin inexplicably increased in MG patients; since there is no known leakage of a heme-containing component in MG, we are exploring whether this is another, previously unrecognized, dysimmune manifestation. We are analyzing the molecular structure of the hemopexin produced in MG for comparison with that produced in leaky-muscle-cell myopathies.

Our electronmicroscopic studies of human thymic epithelial cells from myasthenia gravis patients, directly (regular and pyroantimonate-stained tissue) and after being grown in culture, showed desmosomes and tonofibrils typical of epithelial cells and no muscle-cell like myofibrils contrary to what has been reported by others in cultures of normal animal thymuses (which might have been contaminated with non-thymus muscle cells during tissue removal).

A lymphocyte reaction within the thymus to the proposed "foreignness" of thymic epithelial cells could be of either B- or T-lymphocytes, or both. Our counts of lymphocyte types in fresh MG thymuses as removed at therapeutic thymectomy (histologically lymphoid-hyperplastic) compared with normal thymus demonstrated a slightly increased percent B-lymphocytes, suggesting a reactive increase of them there. Our one-way and two-way mixed reactions with thymic and peripherial-blood lymphocytes from MG patients, compared with control patients, were not abnormal, neither showing evidence of self-stimulation either way nor altered peripheral T-lymphocyte modulation by thymic-lymphocytes (thymic epithelial cells were probably not in our thymic-lymphocyte fractions but may have contaminated those of other studies).

Thymectomy is an established treatment for MG. Our surgical preference continues to be transverse sternal-splitting for adequate removal of thymic tissue (with NIHL) -- our mortality is zero, morbidity low and therapeutic response good to excellent, but often not permanent. More detailed evaluation of our 50 thymectomies since 1968 is underway, correlated with the thymic pathology (with Harvard).

We have now demonstrated that repeat thymectomy can be remarkably beneficial in patients previously improved who subsequently exacerbate and do not respond to medical management. In a number of those patients as well as primary thymectomy patients, we have found positive ^{67}gallium scans -- our thymectomy series is now being correlated with the thymic pathology (with N. M. CC and Harvard). Further, cellular localization of the ^{67}gallium is being done by autoradiography of the excised thymus -- preliminary results suggest the accumulation is in the active lymphoid follicles.

Abnormal lymphocyte function is the pathogenic step presumably suppressed by corticosteroid treatmenet of MG. Confirmed and adopted by most other physicians has been the treatment we introduced to MG, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred). In our own series it continues to be extremely beneficial in the majority of cases, 48 of 52, and for as long as 11 years in a child and 7 years in an adult. Responding best are the older-onset patients, especially the older males. Our 4 non-responders were females, 3 in the menstruating age group. Importantly, though, we have reported that none of our responding patients has become absolved of his requirement for prednisone, even after a gradual tapering of the dose. For example, two patients have exacerbated 3 months after stopping a 5 mg q.o.d. dose (this is about the length of time taken for resumption of synthesis of significant abnormal IgG levels as measured by others in another dysimmune disease). We have modified the treatment slightly by giving, to patients not simultaneously taking anticholinesterase drugs, the single-dose 100 mg prednisone daily for the initial 2-4 weeks before converting to the alternate-day schedule, apparently resulting in more rapid improvement. Fragile patients are not taken off their anticholinesterases but in them prednisone is given in a gradually incrementing single-dose-daily schedule beginning with 10-20 mg. Regarding combining anticholinesterase and prednisone, we reported that low doses of one can be combined with the other advantageously -- but that patients taking both drugs sometimes have a more "brittle" myasthenia and must be watched carefully for ingravescent overdosage by the anticholinesterase. However, because neither anticholinesterase nor prednisone treatment is either curative or completely suppressive, more details of the pathokinesis are needed (v.s.).

Some of our patients have required much higher prednisone doses than 100 mg q.o.d., or even q.d., dosages arrived at empirically in each patient after a period of time. We would like to be able to give a single test-dose and measure parameters of immunosuppression to immediately establish the minimum effective dosage needed, since overdosage, and even therapeutic dosage, produces side-effects of various degrees. Accordingly, the effect of the HSDAD-prednisone treatment on lymphocytes was measured over a 48-hour cycle in a number of MG and other patients, and the results presented. At 6 hrs. after the 8:00 a.m. prednisone dose there is marked depression of T-lymphocyte counts and lymphocyte responses to T-lymphocyte mitogens, and a lesser effect on B-lymphocytes and response to B-lymphocyte mitogens, and there was return of these measurable effects to normal by 24 hrs. after the dose (yet clinically the prednisone has a cumulative beneficial effect over weeks and months). The effects were approximately dose-dependent, establishing that these parameters might answer the original need, but the considerable individual-patient variability means that further precision must be gained.

Our recently-published multistep hypothesis of the pathogenesis of MG, revised on the basis of the blocking factor, bears a striking analogy to the scenario of Verdi's Rigoletto. We therefore term the presumably-detrimental blocking factor a "Sparafucile" molecule after Verdi's hired assassin who without malice killed the unintended victim, in fact, killed the beloved daughter, Gilda (the NMJ-located AChR receptor in MG) of Rigoletto (? malfunctioning helper or suppressor T-lymphocytes), who planned the assassination to be of the Duke of Mantua (? AChR-containing "foreigned" thymic epithelial cell). It is evident from further analysis of the scenario that the answer to MG will only come with the identification and treatment or prevention of Count Monterone's curse (? an environmental factor, e.g., virus).

Our current experience with congenital-persistent myasthenia gravis indicates it is different from the juvenile-onset or adult-onset forms, and probably a different disease. These differences are being codified and will be reported.

The remarkable ancillary benefit that broad-aspect nursing can provide to an MG patient is repeatedly evidenced in our patients -- our multidimensional nursing care approach for myasthenics is available on videotape (with Nursing, CC) and available for general distribution -- this undoubtedly will help improve the care and perhaps save the lives of some myasthenic patients with serious disease, especially in hospitals not frequently caring for such patients. On our own Nursing Unit, MG has been designed as the disease selected for initial development of audit-criteria.

A new electrophysiologic defect of neuromuscular transmission has been found in a patient with a fatigue syndrome and chronic renal disease -- after a brief tetanus there is a diminished but broadened total-muscle action-potential which gradually recovers during rest or slow stimulation, and it is not ameliorated by edrophonium, guanidine, or Ca^{++}. The histochemistry of her muscle biopsy showed marked I-fiber type-predominance but no I-fiber-subtype-grouping, harmonizing with the single-fiber-EMG finding (with Uppsala) of no increased densification of the motor unit -- this was presented.

A new experimental model of neuromuscular junction transmission defect has been found after immunizing animals with peripheral nerve axoplasmic "soluble" protein (see our ALS Project).

The single-fiber EMG technique to study "jitter" and "blocking" phenomena and motor-unit densification, recently described by others, was set up during the visit of E. Stalberg -- it is serving to sharpen our diagnostic and investigative studies in MG and other neuromuscular-disease patients.

Significance to Bio-Medical Research and the Program of the Institute: These findings present new information on the pathologic and pathogenic aspects of myasthenia gravis, and other defects of neuromuscular transmission, on treatment, and on corresponding animal-models.

Proposed Course of Project: To develop more fully the interlinked basic and clinical studies underway directed toward clarification of the pathogenesis and identification of the etiology, and toward elaboration of better means of treatment and prevention.

Publications:

Engel, W. K.: Myasthenia gravis, corticosteroids, anticholinesterases. Ann N.Y. Acad. Sci., 274: 623-630, 1976.

Trotter, J. L., Ringel, S. P., Cook, J. D., Engel, W. K., Eldefrawi, M. E. and McFarlin, D. L.: Experimental autoimmune myasthenia gravis (EAMG): Morphological and immunological studies. Neurology, 1977, in press.

Cook, J. D., Trotter, J. L., Engel, W. K. and McIntosh, C. L.: Altered immunologic cell populations in thymuses from myasthenia gravis patients (MGP). Neurology, 27: 365, 1977.

Engel, W. K., Trotter, J. L., and McFarlin, D. and McIntosh, C. L.: Thymic epithelial cell contains acetylcholine receptor. Lancet, 1: 1310-1311, 1977.

Gershwin, M. E., Glinski, W., Bender, A. N., Ringel, S. P., Steinberg, A. D. and Engel, W. K.: Antibodies to nucleic acids in myasthenia gravis. Int. Arch. Alltergy Appl. Immunol. 51: 245-252, 1976.

Bender, A. N., Ringel, S. P., Engel, W. K., Vogel, Z. and Daniels, M.P.: Immunoperoxidase localization of alpha-bungarotoxin binding -- A new approach to myasthenia gravis. Ann. N.Y. Acad. Sci., 274: 20-30, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01189-09 MN

PERIOD COVERED July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Episodic Weakness and Myotonic Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. King Engel, M.D., Chief, Medical Neurology Branch, NINCDS
OTHER: Roger A. Brumback, M.D., Clinical Associate, MN, NINCDS
N. Bojji Reddy, Ph.D., Guest Worker, MN, NINCDS
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Robert C. Griggs, M.D., The University of Rochester, Rochester, New York
S. Charles Bean, M.D., Wilmington, Delaware

COOPERATING UNITS (if any)

None

LAB/BRANCH Medical Neurology Branch

SECTION --

INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis or are characterized by a significant amount of myotonia. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: plasmalemma, T-system, sarcoplasmic reticulum, myofibrillar complex (i.e. the total excitation-contraction coupling mechanism). With respect to periodic paralysis syndromes, studies are done with agents which are transiently either therapeutic or provocative, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment. The various myotonia disorders are studied with respect to more clearly defining the molecular abnormalities, seeking the underlying pathogeneses and treatment thereof, and finding better ways of symptomatically treating their myotonia. Induced animal-models of myotonia are also used for these purposes.

Serial No. Z01 NS 01189-09 MN

Project Description:

Objectives: To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis or are characterized by a significant amount of myotonia. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: plasmalemma, T-system, sarcoplasmic reticulum, myofibrillar complex (i.e., the total excitation-contraction coupling mechanism). With respect to periodic paralysis syndromes, studies are done with agents which are transiently either therapeutic or provocative, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment. The various myotonia disorders are studied with respect to more clearly defining the molecular abnormalities, seeking the underlying pathogeneses and treatment thereof, and finding better ways of symptomatically treating their myotonia. Induced animal-models of myotonia are also used for these purposes.

Methods Employed: Various techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electronmicroscopy, and biochemical assays of tissue were utilized. Therapeutic trials to raise or lower potassium or sodium and provokative loading tests were used. Acetazolamide was administered as a prophylactic agent for hypokalemic periodic paralysis, and acetazolamide and lithium given to treat myotonia.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have: intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbances; thyrotoxic periodic paralysis; paramyotonia congenita; myotonia congenita; or myotonic atrophy. (Patients with myasthenia gravis are part of another Medical Neurology Branch project.)

Major Findings:

Periodic Paralyses (PP) are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. Associated metabolic abnormalities are known but the actual pathogenic mechanisms are not. Standard palliative preventive therapy in the idiopathic hypokalemic form of PP is potassium, and more recently acetazolamide. Our studies involve improving diagnostic methods, seeking the causes and analysing the pathogenic mechanisms, and improving methods of treatment.

In the hypokalemic form of PP, the treatment we introduced, long-term acetazolamide, has continued to be the best prophylactic agent both for preventing attacks and improving inter-attack weakness. It is now in the textbooks as such. Two of our patients have been treated successfully for more than 11 years. Renal calculi in one of 27 patients, possibly but not definitely related to the acetazolamide, have been the only suspected side-effect. Since muscle does not contain carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown.

Clinical muscle scanning for calcium with ^{99m}Tc-diphosphonate of 3 hypokalemic PP patients did not show any accumulation in muscle during or between attacks, nor any CPK fluctuation.

Myotonia is crippling to various degrees in myotonia congenita and paramyotonia congenita (inherited diseases of unknown cause). A controlled clinical trial showed moderate to remarkable benefit from acetazolamide in 8/10 patients who had failed to respond to, or had been intoxicated by, other anti-myotonia drugs. It remains the long-term treatment of choice in 6 of those patients. A controlled trial of lithium carbonate in one myotonia congenita patient showed decreased myotonia but only at very high levels ($Li > 1.8$ mEq/l).

Myotonic atrophy (myotonic "dystrophy") is an inherited multisystemic disease, with progressive wasting, of unknown pathogenesis. We have previously raised the possibility of at least a partially neurogenic aspect. With our new concept of "myogenous de-innervation", we have now extended that hyothesis to include a possible myogenous muscle plasmalemmal non-receptivity to neural short- and long-term trophic influences.

The model of 20,25 diazacholesterol-induced myotonia of animals was used to evaluate myotonic phenomena. Muscle of the markedly myotonic animals showed: (a) no histochemical changes, (b) no blockage in vivo of the myotonia by α-bungarotoxin, d-tubocurarine, succinylcholine or atropine in contrast to blockage of sciatic-neurectomy-induced fibrillations by all four drugs (and blockage of both myotonia and fibrillations by procaine, tetrodotoxin, KCl or ischemia and of neither by pyridostigmin). Our results demonstrate a major difference, and several similarities, between fibrillation and myotonic discharges -- it is possible to hypothesize that fibrillations and DAC-induced myotonia are mediated through mechanisms involving ionic channels, that both can be produced by activation of junctional/nonjunctional AChRs (or some mechanism coupled to those receptors), but that an unfettered αBT binding portion of the AChR molecule, and an unblocked atropine-binding site, are obligatory only for production of fibrillations. The subcellular localization of tritiated 20,25 diazacholesterol (DAC) is now being analyzed by autoradiography, electronmicroscopic-autoradiography and biochemistry of muscle cell fractions. This, and our showing lack of αBT binding histochemically to muscle fibers in human myotonic diseases, indicate essential differences between myotonic and fibrillating plasmalemmas. Presented was that DAC --induced-myotonic-rat-muscle plasmalemma had 40-60% lower levels of basal and fluoride- and catecholamine-stimulated adenylcyclase activity. Similarly, a 30-60% adenylcyclase decrease was found in 5 patients with myotonic atrophy. These changes could be due to confirmational changes of the enzyme molecule or an altered sterol composition of the plasmalemma.

We confirmed histochemically the finding of an increased number of intrafusal muscle fibers in muscle spindles of myotonic atrophy patients. Our new finding was that muscle spindles are normal in myotonic congenita patients and in rats rendered chronically myotonic with 20,25-diazacholesterol. Therefore, spindle abnormalities in myotonic atrophy perhaps are due to fusimotor deinnervation (? neurogenous myogenous deinnervation) rather than to myotonia per se; moreover, we have found similar spindle abnormalities in rat muscle spindles experimentally denervated (neurogenously).

Significance: These findings present new information on the pathologic and pathogenic aspects of the periodic paralyses and the disorders with myotonia, on their treatment, and on corresponding animal-models.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic period paralysis and the pathogenesis of the disease itself. To seek even better therapeutic agents. To explore the underlying nature of myotonia and seek improved methods of treating it and the underlying disorders.

Publications:

Griggs, R.C., Moxley, R.T., Riggs, J.E. and Engel, W.K.: Effect acetazolamide on myotonia. Ann. Neurol 1: 507, 1977.

Reddy, N.B., Oliver, K.L. and Engel, W.K.: Alterations in the sarcolemmal (SL) adenylate cyclase activity (AC-a) in myotonia. Neurology, 27: 378, 1977.

Brumback, R.A., Bertorini, T.E., Engel, W.K., Oliver, K.L. and Zirzow, G.C.: A comparison of membrane-receptor agents in myotonia and fibrillation in vivo. Neurology, 27: 396-397, 1977.

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Surgical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

A. K. Ommaya, M.D., F.R.C.S., Acting Chief

Summary of Studies in Surgical Neurology Branch

This annual report completes the second anniversary of the incumbent's role as Acting Chief of the Branch. The major change in our research has been a reorganization of a number of research projects in the field of neural trauma and stroke under a new program concerned with the problems of reintegration and regeneration in the central nervous system after varying kinds of insult. This program area will serve to bring together the talents of various Branch members interested in the fundamental mechanisms of neural functions, how they are disturbed by neural trauma and stroke and the mode of their recovery, whether spontaneous or accelerated by various therapies. An important aspect of this program is our basic interest in the mechanisms of the higher integrative cerebral functions in man.

The second major clinical area is the field of malignancies in the nervous system. Our diagnostic abilities continue to be aided by our neuroradiologic colleagues within the Branch and their work on computerized tomography.

The six major areas described in the last annual report summary (July 1975 through June 1976) have been reduced to three major areas as follows:

1. Reintegration and regeneration in the central nervous system.

2. Brain tumors.

3. Diagnostic techniques.

The individual research projects under these three major areas will now be reviewed.

A. REINTEGRATION AND REGENERATION IN THE CENTRAL NERVOUS SYSTEM.

1. Neural Trauma

Our long standing research program in neural trauma has led logically to consideration of the problems of reintegration and regeneration of the central nervous system after trauma. Our published paradigm for the syndromes of cerebral concussion and traumatic unconsciousness has received extensive support from a variety of investigators both nationally and internationally. Recent work on psychological testing after varying grades of head injury as conducted by the Head Injury Center in Galveston, Texas, has provided good data supporting our centripetal theory for the role of shear strains in disconnecting successive layers of neural structures. Validation of the comprehensive finite element mathematical model of the head and neck system continues at the Armed Forces Radiobiology Research Institute, but this work is now secondary to the other projects as described below. The Acting Chief continues to serve as an active member of the Head Injury Data Coordination Group of the D.O.T. and of the Neurotraumatology Committee of the World Federation of Neurosurgeons.

A retrospective study of 100 cases of head injury in which adequate computerized tomography studies had been completed has served to provide the basis for a predictive diagnostic system linking the severity of lesions as diagnosed by computerized tomography with the final outcome as rated by the AIS Scale (abbreviated injury scale) now in common use and approved by the AMA. This predictive scale was presented to the Committee on Neurotraumatology of the World Federation of Neurosurgical Societies as well as to the Research Group on Lesion Outcome Correlation of the Biomechanics Research Group of the Department of Transportation. Both groups agreed to accept this scale as a research tool for further investigations and validation. A key finding in this clinical study of "in-vivo" neuropathology was that the combination of total focal lesion volume estimated by the computerized tomogram plus the intensity of contrast enhancement allowed the production of a Head Injury Severity Index which could be used to predict the severity of the final outcome in surviving patients. Preliminary data would indicate that this prediction could be made in approximately 75 to 80% of the cases.

2. Regeneration of the Spinal Cord after Trauma

In the laboratory we have initiated experiments in the subhuman primate to discover if the report of true spinal cord regeneration (functional and histological) which has recently been reported in dogs can be reproduced in monkeys.

To date six rhesus monkeys have undergone delayed nerve grafting, according to the published techniques. It remains to be seen whether the claimed regeneration can be, indeed, replicated by our data. The clinical arm of this study consists of a preliminary investigation on the extent of blood supply and vascular damage present in cases of established paraplegia in patients. A small group of paraplegic patients will be admitted for selective spinal cord angiography and detailed muscle evaluation which will serve two purposes: one is to establish the extent of vascular damage as compared to the neural damage in such cases, and secondly, this pool of well documented patients will serve to provide candidates for any nerve grafting procedures that may be done if the experiments in the subhuman primates provide positive and conclusive evidence for spinal cord regeneration.

3. Clinical Trial of Microvascular Anastomosis for the Prophylaxis of Stroke in Patients with Transient Ischemic Attacks

This clinical trial, aimed at furthering our knowledge of the mode of neural recovery after vascular insult, is still in progress but is facing a major difficulty related to the access of suitable patients for inclusion in the protocol. Although a large number of potential patients have been screened in the outpatient clinic, a total of only six patients have been admitted in the past year who have proven to be suitable candidates for surgical intervention. It is very likely that this study will be terminated because of difficulties in adequate patient accrual.

4. Disintegration and Reintegration of Higher Cerebral Functions in Man

Our studies of injuries to the head and spinal cord both in animals and man have led to the following hypothesis: The pattern of reintegration of cerebral and spinal functions after trauma mimic the ontogenetic development of those functions in the same species. This hypothesis is a further development on earlier observations made on the recovery of functions after focal lesions in the central nervous system in lower species. It is our suggestion that the ontogenetic recapitulation of functions observed after focal lesions is true also for the effect of diffuse lesions such as seen in head injury and spinal cord injury. This hypothesis is being tested in two ways. The first is a retrospective study of our experience with focal-surgical lesions made in the treatment of patients with epilepsy, pain and involuntary movement disorders. The serial neurological and behavioral data which has accumulated over the past 15 years has not been analyzed from the aspect described above. Secondly, a retrospective analysis of the recovery process in a group of severely head injured patients is also being made. Two prospective clinical studies have been planned to study the mechanism of reintegration functions after trauma which we propose will be analogous to the mode of initial development or learning of functions. The first will be an electrophysiological study of the 3-dimensional organization of cortical columns in human cortex during surgical procedures such as craniotomies for brain tumor and focal epilepsy. The second study uses evoked potential techniques to monitor brain activity during the process of learning a new paradigm; the primary aim here is to study changes in evoked potential distribution during the learning of a new task. This data will be used to test a new theoretical model for global brain function which is being developed in collaboration with the Division of Computer Research and Technology. The task that has been chosen is the learning of a method for control of autonomic nervous system function as described in the next project.

5. The Learning of Hand Temperature Self-Regulation in Alleviation of Headache

This study has been developed primarily to answer the needs for a clinical paradigm whereby patients who are learning control of an autonomic function may be studied to discover the possible neural mechanisms of such learning under controlled conditions. The study consists of a clinical trial of the method of temperature feedback aimed at reducing the frequency and severity of migraine headaches; a second aspect is the simultaneous recording of somatosensory visual and auditory evoked potentials in order to learn what happens at this level of analysis.

B. BRAIN TUMORS

Detailed analysis of our results in over 100 patients with malignant gliomas who have completed trials of adjunctive therapy in our Branch have revealed two important findings: Firstly, there appears to be a direct correlation between the effect of chemotherapy with the grade of the tumor. This has been suggested in earlier studies but only when comparing the response of more benign gliomas (Grade 1 and 2)as compared to the more malignant ones (Grade 3 and 4). We have found that Grade 3 tumors also behave differently from Grade 4 with the former being not significantly improved by chemotherapy and, indeed, our results indicate that adjuvant chemotherapy may have deleterious effects in all grades of glioma other than the most malignant. Our data on the cellular immune responses of patients with such tumors would tend to suggest that the reason patients with less malignant tumors do not benefit from additional chemotherapy is that such chemotherapy serves to depress cellular immune mechanisms and, therefore, reduce long term survival. Because such mechanisms are absent in the most malignant gliomas, chemotherapy cannot have any additional deleterious effect on the anergic immune system. We have also observed in collaboration with the Division of Cancer Treatment, NCI, that the level of immune competence is more severely depressed in patients with brain tumors compared to patients with other types of solid cancers of equivalent volumetric dimensions. This finding is probably of basic importance with regard to the immunologic mechanisms in solid tumors. We are proposing that this selective depression in central nervous system neoplasia may be related to the direct effect of the tumor on the control mechanisms for immunologic responses. We have been able to evoke intracerebral delayed hypersensitivity reactions with acceptable morbidity but with no demonstrable clinical improvement in a small number of patients. It is felt that this approach will probably not be effective until the immune competence of the patient can be restored to a maximum. We are thus proposing a new strategy for combination therapy whereby immune reconstitution agents such as Thymosine and Levamisole will be used in the initial phases of therapy when immune suppressive treatments such as radiotherapy and chemotherapy are being used. By observing T-cell levels (which we have found to be correlated with the integrity of the cellular immune response mechanism) we hope to determine the point at which immune reconstitution agents are no longer required and immune stimulating agents such as BCG may now be used more effectively. This hypothesis was presented at a recent workshop on tumor immunology that was held under the auspices of Duke University and the National Cancer Institute in Chapel Hill, N.C. The current clinical trial of local chemotherapy is now organized as a phase 3 study which is evaluating the specific effects of local 8-Azaguanine given after surgery and radiotherapy. In collaboration with the Pediatric Oncology Branch of the National Cancer Institute, we have developed an assay for 8-Azaguanine which will prove to be of considerable use in developing more rational drug schedules for therapy. Experimentally we have developed a primate model for chronic intrathecal studies which is proving of great value in physiologic, pharmacologic and chemotherapeutic studies. In collaboration with the Arthur V. Davis Center for Behavioral Neurobiology, San Diego, California, this model is also being used to evaluate the effects of endorphins in the rhesus monkey CSF. We have also demonstrated in our murine glioma model that the MER fraction of PPD as well as C. parvum are not effective in enhancing survival. Current experimental studies are aimed at development of a human glioma model grown in nude mice which will be ideal for a number of experiments including a direct test of our hypothesis for central control of immune mechanics by direct central nervous system stimulation in animals.

C. DIAGNOSTIC TECHNIQUES

The main technique which is being developed by the Section on Neuroradiology and Computed Tomography in our Branch has been in computer assisted tomography. A large number of voluntary normal control patients have been studied and cases of demyelinating degenerative processes are being evaluated. Patients with Parkinsonism and Huntington's disease are also being studied as well as patients with postradiation necrosis of the brain. Evaluation of the spinal cord with the CT technique and the development of a new technique of computerized assisted myelography have been unique contributions of this Section. Technical advances in this area have been the demonstration of the feasibility of simultaneous dual energy scanning using an original split detector. In addition, it has been shown that protons can be used to detect differences in physical properties in material at the 0.1% level as compared to the 0.5% level in present commercial scanners. Further development of spinal cord angiography using both radiographic and isotopic techniques has continued as well as further studies extending our knowledge of the CSF system using isotope ventriculography and cisternography.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 00907-16 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Reintegration and Regeneration After Trauma to the Brain and Spinal Cord in Man and Animals
(Previous Title: Head Injury)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.	A. K. Ommaya	Actg. Chief, Surg. Neurol. Br.	SN	NINCDS
Other:	P. Kochie	Med. Off. (Neuropathologist)	SN	NINCDS
	N. Gunby	Neurosurgeon	NR	AFRRI
	D. O. Davis	Assoc. Chairman Dept. Radiol. George Washington Univ. Med. Center, Washington, DC	R	GWUMC
	M. Mendhi	Visit. Associate	SN	NINCDS
	H. Rizzoli	Chairman, Dept. of Neurosurgery G.W.U.M.C., Washington, D.C.	NS	GWUMC
	L. Riddick	Dept. Medical Examiners D.C. General Hospital Washington, DC	ME	DCGH

COOPERATING UNITS (if any) Dept. of Neurobiology, Armed Forces Radiobiology Research Institute; Dept. of Radiology, George Washington University Medical Center, Washington, D.C., Dept. of Neurosurgery, G.W.U.M.C., Medical Examiners Office, DC General Hospital, Washington, DC.

LAB/BRANCH
Surgical Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	3.5	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three experimental models of neural trauma are being studied. (1) Inertial loading in a subhuman primate validating a mathematical model which will be used for developing improved standards for head injury protection and prevention. (2) A series of studies on isolated neural tissues correlating the primarily neural effects of the trauma independent of the vascular system. (3) A model is of spinal cord injury. This is a modification of a recently published spinal cord crush injury developed in the dog which we have adapted for the rhesus monkey. We are evaluating the regenerative capacity of the primate spinal cord in this model using peripheral nerve and cultured Schwann cell grafts with and without additional therapy using pulsed radiofrequency energy. Clinical studies on neural trauma are being carried out in collaboration with GWUMC aimed at correlating the in-vivo neuropathology as seen on C.T. scans in head injury patients with the clinical data and in selected cases with the post-mortem neuropathology. Selected patients with paraplegia are also being evaluated for extent of neural and vascular damage.

PROJECT DESCRIPTION:

Objectives:

1. To understand the mechanisms of mechanical trauma to the nervous system and its reintegrative and regenerative responses to grades of such trauma.

2. To design rational methods for protection, prevention, diagnosis, prognosis and treatment of trauma to the brain and spinal cord.

3. To develop a therapy aimed at promoting regeneration and reintegration in neural tissues after trauma with special reference to spinal cord injury.

METHODS:

1. In man, investigate and possibly separate the vascular component from the neural of the damage sustained in cases of paraplegia and quadriplegia after spinal cord injury in preparation for consideration of surgical therapy aimed at regeneration of spinal cord function.

2. Using the spinal cord crush technique, delayed peripheral nerve grafting is being evaluated with and without adjuvant pulsed radiofrequency energy therapy in subhuman primates.

3. Utilizing programmable head accelerating devices, subhuman primates are subjected to controlled head accelerations to obtain reproducible injury at three levels; subconcussive, concussive with no sequelae and concussive with sequelae.

4. Utilizing O_{15} a study of cerebral blood flow and metabolism at varying levels of brain injury in our primate model is being developed. This study will compare the flow and metabolic changes with alterations in neurophysiologic functions as measured by somatosensory, visual and auditory evoked potentials. Correlation of these data with behavioral and pathologic data will enable a clear analysis of the primary and secondary effects of neural trauma.

5. Utilizing computed tomography, the serial noninvasive evaluation of head injury in our primate model will be carried out. These data will be correlated with similar data from head injured patients.

6. A study of the dynamics of brain swelling and brain edema in our head injury model using neurochemical and physiologic techniques has also been initiated.

MAJOR FINDINGS:

Based on the experimental work of the past 5 years a hypothesis for cerebral concussion has been developed and published. This hypothesis is able to explain for the first time both the paralytic and amnesic phenomena of cerebral concussion and predicts the possible mechanisms for post-traumatic sequelae. Data produced from clinical observations and from our experimental model in the monkey are in general support of this hypothesis to date. The experimental methodology and research design leading to our current hypothesis have proven to be very successful and have been copied by the Head Injury Center at the University of Pennsylvania. Our hypothesis also predicted certain pathological findings for head injury in man which have recently been confirmed by Dr. Hume Adams in a report on fatal head injuries in Glasgow.

Our hypothesis has also served as a predictor for the finding of subdural hematomas in battered children where no evidence of direct head impact has been found. Pediatricians treating this condition have accepted the mechanism which we have proposed; namely, indirect accelerative effect on the head produced by shaking the child, which thus results in large subdural hematomas without any evidence of direct blows to the head. Our work with computed tomography for head injury research has established the basis for correlating linear attenuation coefficients in vitro with the data found in vivo in patients. This study is being done in collaboration with the Georgetown University Medical School. In our spinal cord injury work we have confirmed the significance of the cavitation response in affecting regeneration.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

1. Our work is continuing to provide the standard criteria nationally and internationally for experimental studies in neural trauma research.

2. Correlation of neurophysiologic (evoked potential), physiologic (cerebral blood flow and metabolism) and pathologic data in our experimental models will enable us to separate the primary and secondary effects of neural trauma.

3. Validation of a mathematical model for head injury will enable significant improvements in head injury protection and prevention strategies.

4. Development of our spinal cord injury work may contribute to a cure for paraplegia as well as aid in the treatment of other neural trauma.

PROPOSED COURSE:

1. Develop our combination grafting/R.F. mode of treatment for paraplegia in monkeys.

2. Determine the vascular injury component in cases of spinal cord injury in man.

3. Apply the data derived from 4 and 5, below, to the treatment of human paraplegia and quadriplegia.

4. Develop the techniques of evoked potentials and C.A.T. as clinically practical, serial, noninvasive methods for diagnosis and prognosis of neural trauma severity. C.A.T. will be used to measure volumetric and blood brain barrier changes in the brain, both in animal model and in patients after head injury.

5. Test new therapies aimed at blocking secondary responses to trauma and accelerating neural reintegration after injury.

PUBLICATIONS:

1. Ommaya, A.K.: Spinal fluid fistulae. In Keener, E.B. (Ed.): Clinical Neurosurgery. Proceedings of the Congress of Neurological Surgeons, Atlanta, Georgia, 1975. Baltimore, Williams and Wilkins Co., 1976, chapt. 27, pp. 363-392.

2. Ommaya, A.K. and Gennarelli, T.A.: A physiopathologic basis for non-invasive diagnosis and prognosis of head injury severity. In McLaurin, R.L. (Ed.): Head Injuries: Second Chicago Symposium on Neural Trauma. New York, Grune & Stratton, 1976, pp. 49-75.

3. Ommaya, A.K., Murray, G., Ambrose, J., Richardson, A. and Hounsfield, G.: Computerized axial tomography: estimation of spatial and density resolution capability. Br.J.Radiol. 49: 604-611, 1976.

4. Advani, S.H., Ommaya, A.K. and Yang, Wen-Jei: Head injury mechanisms: Characterizations and clinical evaluation. In Ghista, D. (Ed.): Biomechanics with Applications to Physiologic Monitoring. New York, Marcel-Dekker, Inc., 1976. In press.

5. Wyatt, R.J., Cantor, F., Gillin, J.C., Gordon, E., Karoum, F., McCullough, D., Neff, N., Ommaya, A., Rauscher, F.P., Seaborg, J.B. and Slaby, A.: Ventricular fluid metabolites of phenolic amines and catecholamines. In Usdin, E. and Sandler, M. (Eds.): Trace Amines and the Brain. New York, Marcel-Dekker, Inc., 1976, pp. 209-231.

6. Wood, J.H., Parver, M., Doppman, J.L. and Ommaya, A.K.: Experimental intraoperative localization of retained intracerebral bone fragments using transdural ultrasound. J.Neurosurg. 46: 65-71, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01025-15 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Tumors of the Nervous System

LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	A. K. Ommaya	Act. Chief, Surg. Neurol.	SN/NINCDS
OTHER:	P. Kochie	Neuropathologist	SN/NINCDS
	P. Chretien	Senior Investigator	SURG/NCI
	C. H. Kent	Radiation Oncologist	DCT/NCI
	D. Poplack	Pediatric Oncologist	PO/NCI
	M. Mendhi	Visiting Associate	SN/NINCDS
	D. Sadowsky	Statistician	OBE/NINCDS

COOPERATING UNITS (if any)
Laboratory of Experimental Chemotherapy, Division of Cancer Treatment, Drug Research & Development; Surgery Branch, NCI; Radiation Oncology and Pediatric Oncology, NCI

LAB/BRANCH
Surgical Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A controlled clinical study of the effect of intratumoral chemotherapy using 8-Azaguanine compares this method given in combination with systemic CCNU after surgery and radiotherapy to the effect of adjuvant chemotherapy with CCNU given alone. In a pilot study our methods have increased the mean survival time to approximately twice that reported by other centers using CCNU alone after surgery and radiotherapy. An experimental human glioma model in the nude mouse is under development and this model will be used for chemotherapeutic and immunotherapeutic studies. Dosage of therapeutic agents used for local intrathecal chemotherapy is being monitored in a primate model for chronic intrathecal chemotherapy. Our immunologic approach is currently aimed at testing the necessity for immune reconstitution with thymosine (in conjunction with chemotherapy) prior to immune stimulation with BCG.

Project Description:

Objectives:

1. To develop surgical and immunochemotherapeutic methods for the treatment of human malignant gliomas and other "inoperable" tumors of the nervous system.

2. To evaluate immunologic parameters of patients undergoing therapy of brain tumors.

3. To develop animal models suitable for evaluation of immunotherapy and chemotherapy trials.

4. To evaluate the a) immunological identification of tumor-specific antigens; b) chemical makeup of tumor-associated antigens, initially assumed to be in glycoproteins isolated from tumor cell membranes; c) antigenicity of tumor-associated glycoproteins.

5. To improve the quality and quantity of survival of patients with malignant brain tumors.

Methods Employed:

1. Microneurosurgical and intravital staining techniques to enhance maximal reduction of tumor cell mass. We are currently investigating the use of Tantalum suspensions for this study.

2. Patients with histologically verified glioblastoma multiforme and malignant astrocytomas (Grades III and IV) are selected. The extent of neurological deficit and intracranial mass anatomy is established clinically and neuroradiologically, including evaluation by computerized axial tomography before and after maximal tumor resection and treatment with radiotherapy.

3. Cerebrospinal fluid reservoirs and intratumoral cysts are inserted to allow evacuation of tumor bed contents and for infusion of chemotherapeutic agents or agents to induce intratumoral delayed hypersensitivity reactions.

4. Patients are randomized into a prospective controlled study to evaluate combined chemotherapy with CCNU and 8-Azaguanine versus chemotherapy with CCNU alone. (Both groups receive the tumor cyst implant).

5. The program of combined chemotherapy utilizes oral CCNU, 130 mg/sq meter body surface, and intratumoral 8-Azaguanine, 100 mg. by infusion, the oral drug being given for six doses, one dose per 6-8 week period or until onset of liver or marrow disturbance. The intratumoral drug is given once a week for six weeks and then once a month for one year, and once a month indefinitely after that.

6. A murine glioma model has been developed which can reliably induce intracerebral tumors in mice and provide large numbers of cells for immunotherapy of that tumor. This animal model was also used to test varying combinations of immunotherapy, chemotherapy, radiotherapy and to check the effect of splenectomy on tumor growth with and without therapy.

7. A model of human glioma in nude mice is being developed.

8. A model for chronic intrathecal chemotherapy in the rhesus monkey has been developed and studies of methotrexate and 8-Azaguanine toxicity are now under way.

9. Serial measurement of T lymphocytes in tumor patients is being used to assess immune competence.

Major Findings:

We have reviewed our experience in 106 patients with cerebral glioma. Our combination therapy with extensive surgical resection controlled by microsurgical technique, followed by radiotherapy and chemotherapy with oral CCNU and intratumoral 8-Azaguanine, has resulted in mean survival times of 93 weeks for grade 4 malignant gliomas. This result is more than twice as good as any published series.

This pilot study is encouraging when one compares our results with mean survival time of less than 40 weeks for conventional therapy (surgery plus radiotherapy) as available in historical data. The NCI Brain Tumor Study Group has conducted a controlled study of the effect of surgery plus chemotherapy with CCNU after radiotherapy, and the mean survival time of this study was only 48 weeks. Our current controlled study is designed to test the hypothesis that our method holds promise for survivals of longer duration with better quality. Our primate model for intrathecal chemotherapy is proving to be of great value for the study of drug kinetics and toxicity in the CNS.

Proposed Course:

1. To complete the clinical trial as proposed. To develop a practical intravital staining method in humans in order to facilitate near total surgical removal of gliomas. To develop the optimum local chemotherapy in our primate model used in conjunction with studies in our murine model.

2. Confirmation of our local chemotherapy regimen would enable patients with malignant glioma to live much longer in good quality survival.

3. If the model of the human glioma in the nude mouse proves to be successful, we will pursue a study of various agents to produce maximum reduction of the glioma using intratumoral and local techniques.

4. The significance of vascular factors in brain neoplasia will be correlated with the immunologic data.

Publications:

1. Albright, L., Seab, J.A. and Ommaya, A.K.: Intracerebral delayed-hypersensitivity reactions in glioblastoma multiforme patients. Cancer 39 (3): 1331-1336, 1977.

2. Wood, J.H., Poplack, D.G., Bleyer, W.A. and Ommaya, A.K.: Primate model for long-term study of intraventricularly or intrathecally administered drugs and intracranial pressure. Science 195: 499-501, 1977.

3. Rajjoub, R.K., Wood, J.H. and Ommaya, A.K.: Granulomatous angiitis of the brain: A successfully treated case. Neurology 27: 588-591, 1977.

4. Spiegel, A.M., DiChiro, G., Gorden, P., Ommaya, A.K., Kolins, J. and Pomeroy, T.C.: Diagnosis of radiosensitive hypothalamic tumors without craniotomy. Endocrine and neuroradiologic studies of intracranial atypical teratomas. Ann. Intern. Med. 85: 290-293, 1976.

MITHSONIAN SCIENCE INFORMATION EXCHANGE ROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02201-02 SN

PERIOD COVERED
October 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Surgical Prophylaxis for Stroke

LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. K. Ommaya	Actg. Chief, Surg. Neurol.	SN/NINCDS
OTHER:	R. Glasser	Staff Radiologist	DR/CC
	A. E. Jones	Asst. Chief, Nuclear Med.	NM/CC
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	C. Horwitz	Senior Investigator	HE/NHLI
	D. Sadowsky	Math. Statistician	OB/NINCDS
	P. Fedio	Research Psychologist	CN/NINCDS
	C. Ajmone Marsan	Chief, Clin. Neurosci.	CN/NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Surgical Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a randomized, controlled clinical trial of microneurosurgical anastomosis between the superficial temporary artery and the middle cerebral artery for prevention of stroke in patients with transient ischemic attacks related to the anterior cerebral circulation. Patients with such symptoms are admitted for workup which includes angiography and EMI Scan and isotope studies to quantify the blood flow in the brain. Randomization between a medical arm and a surgical arm are done after diagnosing the presence and severity of transient ischemic attacks. To date approximately 120 patients have been screened in the outpatient department for this project and only 6 were found suitable for the study. In view of this low access rate, it has been decided to discontinue this clinical trial.

Project Description:

Objectives:

It is well established that the development of an adequate collateral circulation is the key factor in enabling patients to tolerate severe occlusive lesions of the cerebral circulation. Strokes occur when age and atherosclerosis render available collateral pathways inadequate. Approximately 90% of all strokes are occlusive and of these 50-80% have brief warning episodes of diffuse or focal reversible neurologic deficit, i.e. transient ischemic attacks (TIA).

A number of neurosurgeons have demonstrated the feasibility of a technique for increasing collateral CBF to the anterior circulation by anastomosing the superficial temporal artery (STA) to a cortical branch of the middle cerebral artery (MCA). This technique has now been well established as a practical surgical technique, with post-operative patency rates for the STA-MCA bypass exceeding 90% and with clinical, CBF and psychometric data showing significant improvement over preoperative levels. To date, however, no controlled study of this type of prophylactic surgery for stroke has been carried out.

This project seeks, therefore, to control the medical management and test the variable of STA-MCA surgery by randomizing it in a series of patients with anterior circulation TIA receiving uniform medical therapy. Sequentially ordered groups of patients receiving either medical therapy or medical plus surgical therapy will be compared for TIA and stroke incidence, pre- and post-treatment clinical status, cerebral blood flow changes and psychometric performance.

Methods Employed:

1. Clinical Testing. Detailed quantitative neurological testing will be performed before and at specified intervals after the initiation of treatment.

2. Electrophysiologic Testing. Serial electroencephalography will be carried out during outpatient and inpatient attendance. Averaged evoked potential assay of somatosensory, visual and auditory functions will be carried out before and after the treatment period at the time of the CBF estimations.

3. Isotope Scanning. Isotope scans will be carried out before and after the treatment. Post-treatment scans will be done 3 months and 12 months after initiation of therapy.

4. Computerized Tomography (EMI Scan). CT scans before and after treatment will be obtained. Ventricular capacity and visible lesions will be recorded in terms of volume using a video planimeter developed in the Television Engineering Branch of the Clinical Center. Lesions will be identified visually as well as by checking for numerical deviations from the norm in terms of recorded linear attenuation coefficients.

5. Angiography. Cerebal pan angiography via a transfemoral catheter will be obtained once before and after treatment initiation. The posttreatment angiogram will be obtained prior to patient discharge but not less than 2 weeks after treatment initiation.

6. Cerebral Blood Flow Measurement. This will be carried out before and after treatment, immediately before and after the angiography. A third estimate will be made one year after the initiation of the treatment. The technique of CBS measurement will be the noninvasive method of Austin et al. using a bolus of 10-15 mc of ^{133}Xenon injected intravenously as modified in the Nuclear Medicine Department of the Clinical Center.

7. Psychometric and Social Evaluation. The patient's psychologic and social status will be evaluated before, one month after and one year after the treatment by a clinical psychologist and a social worker.

8. Each patient will be assigned to one of two categories, the first receiving continuing medical therapy alone, the second receiving a TCA-MCA anastomosis plus medical and appropriate supportive therapy.

9. Surgical Technique. This will be as described by Reichman and others.

Major Findings:

To date approximately 120 patients have been screened in the outpatient department for this project and only 6 were found suitable for the study. In view of this low access rate it has been decided to discontinue this clinical trial.

Publications: None

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ITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02253-01 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Clinical Efficacy of Hand Temperature Self-Regulation in Alleviating Migraine Headache

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. L. Frankel	Staff Investigator	SN NINCDS
Other:	A. K. Ommaya	Acting Chief	SN NINCDS
	P. Fedio	Psychologist	CN NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Surgical Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to evaluate rigorously the clinical efficacy and specificity in migraine headache of operantly learned hand temperature control via skin temperature biofeedback. Several patients have begun such biofeedback-assisted training or noninstrumented autogenic relaxation training, while most others are still in the baseline period monitoring headache activity.

Project No. Z01 NS 02253-01 SN

Project Description: Previous studies have reported biofeedback techniques to be clinically effective in alleviating migraine headache. These studies, however, have generally lacked methodological features essential for substantiating such effectiveness. This study was undertaken to assess rigorously the clinical efficacy and specificity in migraine headache of operantly learned hand temperature control via skin temperature biofeedback.

Methods Employed: Outpatients meeting several diagnostic and symptom frequency criteria for classic or common migraine headaches are randomly assigned to one of three groups: (1) biofeedback-assisted handwarming; (2) biofeedback-assisted handcooling; and (3) verbal relaxation training. Patients in groups 1 and 2 will undergo skin temperature feedback training (from middle finger) to learn to warm and cool their hands at will respectively; those in group 3 will learn deep relaxation via the verbal techniques of autogenic relaxation training.

All patients will come to the laboratory for 18 training sessions over an 11-week period. Each patient will practice twice daily at home and keep a log of the practice. Aspects of headache activity, such as frequency, severity, duration, drug use, etc. will also be recorded daily throughout the study period starting at least eight weeks prior to training and at regular intervals in the one-year followup period.

Major Findings: Of the eight patients having entered the study so far, three are in various phases of hand temperature training or autogenic relaxation training; the others are recording headache activity in the baseline period. Preliminary results from the three patients in training suggest that a meaningful reduction in headache activity is associated more with learning to handwarm than with autogenic relaxation.

Significance-to Biomedical Research: If the patient's clinical efficacy for migraine headache is demonstrated, voluntarily controlled changes in autonomic nervous system activity and their cognitive and emotional correlates may have widespread potential application in other disorders where psychologic factors influence symptom activity (e.g. epilepsy, tension headaches, muscular dysfunctions etc.).

Proposed Course: Although originally designed as a 3 to 5 year study, this project will be terminated at the end of July, 1977 as a result of the unanticipated resignation from the NIH of the principal investigator.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01047-15 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Isotope-Ventriculography and Isotope-Cisternography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Di Chiro	Head, Section on Neuroradiology & CT	SN NINCDS
	M. K. Hammock	Senior Staff Fellow	SN NINCDS
OTHER:	G. S. Johnston	Chief, Nuclear Medicine Dept.	NM CC
	A. E. Jones	Assistant Chief	NM CC
	M. V. Green	Chief, Applied Physics Section	NM CC

COOPERATING UNITS (if any) Nuclear Medicine Dept., Diagnostic Radioisotope Section, CC, NIH

LAB/BRANCH
Surgical Neurology Branch

SECTION
Section on Neuroradiology and Computed Tomography

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: .2	PROFESSIONAL: .2	OTHER: .0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Isotope-ventriculography and isotope-cisternography are diagnostic tools permitting the morphologic and dynamic study of the cerebrospinal fluid pathways more accurately than has ever been possible with any other diagnostic test.

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictoral outline of the ventricular system (isotope-ventriculography) and of the subarachnoid intracranial spaces (isotope-cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospinal fluid itself will be obtained. The spinal CSF spaces may also be evaluated.

Methods Employed: The radioisotope cisternography and ventriculography procedures are now well established.

Recently we have devoted particular attention to one aspect of the CSF flow, i.e., its descent to the spinal subarachnoid space. Experiments have been carried out in the Rhesus monkey after injection of radiopharmaceuticals within the ventricular system. The scintiphotographic data are appraised with the assistance of a computer. Digital analysis is performed using a small dedicated computer (HP 5407A Hewlett Packard Scintigraphic Data Analyzer), mated to the Anger camera. For this purpose the pin-hole collimator is positioned so that the entire lateral length of the cerebrospinal space is within the field of view of the Anger camera detector. Nine regions of interest cursors are drawn: one over the cerebral convexity, and one to include the entire spinal subarachnoid space. Time-activity curves are obtained from each region of interest, simultaneously without moving the animal for the next three and one-half hours.

Preliminary experience has been gained in clinical material on the descent to the spinal subarachnoid space of the CSF. Many patients, all newborn infants, and all with abnormalities of the CSF circulation--the majority was made up of cases of myelomeningocele--have been subjected to the following procedure. A radiopharmaceutical has been injected into the lateral ventricles of the brain and the descent of the cerebral spinal fluid into the spinal canal has been studied with an Anger camera, and in selected cases with the help of a computer. When the computer has been used, total gathering of the data has been attained above the entire spine for a period of at least one hour following the intraventricular injection. A number of patients affected by meningeal leukemia, and in whom radioisotope ventriculography was carried out, were also followed up with spine scanning for the purpose of gaining experience with the spinal CSF descent.

Major Findings: None

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular we now have a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoid pathways.

Last year's investigations should have practical implication for the diagnosis and followup of such conditions as porencephaly and hemispheric glioma.

The CSF spinal descent studies should enable us to determine what is the importance of the spinal CSF route of flow as an alternative pathway of resorption. The observations of the spinal descent pattern of the CSF have also heuristic significance in regard to a possible analysis of metabolites and drugs distribution through the CSF from the endocranial cavity to the spinal theca.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal pathologic flow of the CSF will be gathered by the techniques of isotope-cisternography and isotope-ventriculography.

Publications: None

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PROJECT NUMBER

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MITHSONIAN SCIENCE INFORMATION EXCHANGE .ROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01195-13 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Radiographic and Radioisotopic Angiography of the Spinal Cord

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Di Chiro	Head, Section on Neuroradiology and Computed Tomography	SN NINCDS
OTHER:	J.L. Doppman	Chief	DR CC
	J.R. Herdt	Deputy Chief	DR CC
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	L. Wener	Chairman, Dept. of Radiology, Greater Southeast Community Hospital	
	G.S. Johnston	Chief	NM CC
	A.E. Jones	Assistant Chief	NM CC
	M.M. Mishkin	Professor of Radiology, Hospital of University of Pennsylvania	

COOPERATING UNITS (if any) Diagnostic Radiology Dept., CC, NIH; Dept. of Radiology, Greater Southeast Community Hospital, Wash., DC; Nuclear Medicine Dept., CC, NIH; Dept. of Radiology, Hosp. of the Univ. of Pennsylvania, Phil., PA; Medical Examiner's Office, City of Philadelphia, Dept. of Public Health, Phil., PA.

LAB/BRANCH
Surgical Neurology Branch

SECTION
Section on Neuroradiology and Computed Tomography

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Selective arteriography (radiographic) of the spinal cord is a diagnostic technique which has proven to be very informative in cases of arteriovenous malformation, tumor, obstructive vascular disease, trauma, and postradiation damage of the spinal cord.

Radioisotope angiography of the spinal cord offers distinct advantages as a screening method, and in certain types of intraspinal pathology may give information not available by any other diagnostic test.

Preliminary experience with computed tomography of the spine indicates that this new methodology may be useful in the evaluation of certain vascular lesions of the spinal cord.

Project Description:

Objectives: The introduction of cerebral angiography (1927) has markedly increased our knowledge of the vascular pathology of the brain. The vascular pathology of the spinal cord, on the other hand, remains a largely unexplored area.

Since 1964 we have been carrying out angiographic studies of the spinal cord and developed this technique into a reliable diagnostic tool. Selective injection of the contrast medium has made the difference between an occasional demonstration, and the consistent visualization of the spinal cord vasculature.

The usefulness of selective arteriography in cases of spinal cord arteriovenous malformations is now well established. We are continuing to use this technique to: 1) Learn more about the pathophysiology of the spinal cord arteriovenous malformations so that a better treatment of these important and frequent lesions may be developed. 2) Evaluate how useful spinal cord angiography is in cases of spinal cord tumors. 3) Establish whether or not this technique can be of diagnostic value in the study of obstructive spinal cord vascular disease. 4) Assess the usefulness of this technique in intervertebral disc pathology. 5) Evaluate the diagnostic possibilities of this procedure in posttraumatic spinal cord injury with or without vertebral fractures. 6) Establish the value and limits of newly introduced radioisotopic angiography of the spinal cord. 7) Explore the possible emergency therapeutic means which could be employed to treat and cure, or at least minimize the effects of the dreadful postangiographic cord complications. 8) Acquire new information regarding the fine vasculature of the human spinal cord, with particular emphasis on the intrinsic vessels (sulcal or central arteries and other perforating or penetrating branches). This goal is accomplished by post-mortem microangiographic techniques in cadavers of all age groups. We are paying particular attention to cords of aged adults.

Methods Employed: Selective arteriograms with modern catheter techniques are carried out in patients in whom spinal cord vascular or tumoral lesions are suspected. The subtraction technique is used to better visualize the injected vessels. In addition, in the last fiscal year we have gained considerable experience with the direct radiographic magnification angiograms.

For the technique of radioisotope angiography of the spinal cord a bolus of 15 mCi of ^{99m}Tc human serum albumin (1 to 2 ml) is injected in a left antecubital vein. Immediately afterwards, cinescintiphotographic or rapid flow Polaroid views of the various segments of the spine are abtained with an Anger scintillation camera. In the last fiscal year our scintiphotographic data have been significantly ameliorated by a computer assisted analysis and reconstruction of images, as well as by isometric contour computer display of the data.

For the technique of computed tomographic angiography of the spinal cord we use a computed tomography (CT) body scanner and we carry out timed serial tomograms of the area of interest of the spine after the intravenous introduction of angiographic contrast medium.

For the post-mortem studies of the vessels of the human spinal cords, (aged adults) we have used our previously developed microangiographic techniques.

Based on the observation made elsewhere, that in two patients who died soon after aortography with spinal cord complications, the iodine content in the CSF was enormously increased, we are attempting an emergency therapeutic method consisting of flushing out the "iodine contaminated" CSF.

Major Findings: We have continued to accumulate experience in the areas of:

1) Selective arteriography in cases of herniation of thoracic discs.

2) CSF lavage in patients who develop symptoms and signs of cord involvement after abdominal aortography or other types of arteriographic studies.

3) Post-mortem microangiographic evaluation of the aged human cord.

The new development in the current fiscal year has been the introduction of computed tomography (CT) in the analysis of vascular diseases of the spinal cord. We have gathered preliminary experience in six patients with arteriovenous malformation of the spinal cord using a CT scanner. We have been able to recognize the pathological vessels of two of the arteriovenous malformations. In one case, in which occlusion of the pathological vessels was obtained through percutaneous embolization, we were able to demonstrate the therapeutic attempt.

Significance to Bio-Medical Research and the Program of the Institute: Radiographic and radioisotopic angiography of the spinal cord are increasing our understanding of the large group of conditions in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: Post-mortem microangiography of the aged adult's cords should offer new insights on such conditions as obstructive vascular disease of the cord due to arteriosclerosis and cervical spondylosis, and possbily on degenerative and demyelinating cord diseases.

We are "watching" for possible further technical developments of the technique of selective arteriography of the spinal cord. We have recently established the value of direct radiographic magnification, and we are considering initiating the use of angiotomography for a better visualization of the smaller vessels, possibly the intrinsic arteries and veins of the cord.

Improved x-ray vascular contrast media will also enhance the diagnostic possibilities of spinal cord angiography. We are following very closely the recent developments in the area of polimeric, ion-balanced and non-ionic iodinated x-rays contrast media.

We will dedicate much of our attention to technical improvements in the newly introduced radioisotope angiography of the spinal cord. This method, which we are extensively using as a screening and followup procedure, could become a more definitive and informative diagnostic examination. By increasing our resolution through a computer-assisted reconstruction and enhancement of the images, we should be able to extract a lot of diagnostic information from this simple and innocuous technique.

Computed tomographic angiography of the spine and spinal cord represents one of the areas in which we will concentrate a great deal of interest.

Publications: Di Chiro, G.: Recognition of radiculomedullary arteries. (Letter to the Editor) Brit. J. Radiol. 49: 651-652, 1976

Di Chiro, G., Doppman, J.L. and Wener, L.: Computed tomography of spinal cord arteriovenous malformations. Radiology 123: 351-354, 1977

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01654-10 SN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Experimental Spinal Cord Angiography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Di Chiro	Head, Section on Neuroradiology &CT	SN NINCDS
OTHER:	M. K. Hammock	Senior Staff Fellow	SN NINCDS
	J. R. Herdt	Deputy Chief, Diag. Radiology Dept.	DR CC
	J. Fein	(formerly of Armed Forces Radiobiology Research Institute, Bethesda, MD)	
	K. Earle	Chairman, Dept. of Pathology	USUHS

COOPERATING UNITS (if any) Diagnostic Radiology Dept., CC, NIH; Armed Forces Radiobiology Research Institute, Bethesda, MD; Uniformed Services University of the Health Sciences, Bethesda, MD.

LAB/BRANCH

Surgical Neurology Branch

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental spinal cord angiography in the Rhesus monkey is increasing our understanding of the blood supply of the spinal cord both in physiological and pathological conditions.

Project Description:

Objectives: The clinical value of the NIH developed technique of selective arteriography in the management of arteriovenous malformations and tumors (in particular hemangioblastomas) of the spinal cord is now well established.

In order to expand the clinical applications of arteriography of the spinal cord we are working with experimental angiographic and microangiographic models in primates.

Previously, we have concentrated our attention on the area of experimental obstructive vascular disease of the spinal cord in the Rhesus monkey. In the past fiscal year much of our experimental investigation has delt with an iatrogenic pathological condition. Postradiation myelomalacia (myelitis).

In the area of postradiation myelitis we are particularly interested in establishing whether the basic pathological lesion of this dreadful complication is primarily neurogenic or vascular.

Methods Employed: Preradiation angiographic studies (selective technique) of the thoracolumbar segment of the spinal cord are carried out in young, healthy Rhesus monkeys. Soon after, selective irradiation of the thoracolumbar cord using the LINAC accelerator (A.F.R.R.I.) is initiated. Total dosage and modalities of delivery are chosen to approximate the radiation protocol which most often seems to cause myelomalacia in human patients.

At the end of the radiation, the monkeys are kept under careful observation for periods of many months. Neurological testing of the lower limbs is performed twice a week. If and when the monkeys show signs of developing or established paraplegia, repeat selective arteriography of the irradiated segment is carried out. Following this, the animals are perfused for microangiography of the spinal cord and then sacrificed. The cord is studied by gross observation, microangiography, routine histology and special myelin stains. Careful gross and histological analysis of the neighboring aortic segment, its branches and the pertinent radiculomedullary arteries is also carried out.

Major Findings: We are on the course of evaluating the pathological changes of the spinal cord from monkeys in which we successfully induced postradiation paraplegia (myelopathy).

Significance to Bio-Medical Research and the Program of the Institute: We should be able to shed some light on the pathogenesis of the postradiation myelitis. This is not a rare complication in human patients (over 500 cases have been reported in literature).

Proposed Course of Project: Appraisal of the postradiation data which we have already collected as well as new data in other irradiated animals now under observation. We will attempt to study (by angiography and microangiography) human patients (or human specimens) with postradiation spinal cord damage.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01791-09 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Angiography of Cerebral Venous Obstructive Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Di Chiro	Head, Section on Neuroradiology & CT	SN NINCDS
	M. K. Hammock	Senior Staff Fellow	SN NINCDS
OTHER:	K. Earle	Chairman, Dept. of Pathology	USUHS

COOPERATING UNITS (if any) Uniformed Services University of the Health Sciences, Washington, D.C.; Experimental Surgery and Clinical Medicine Section, Veterinary Resources Branch, Division of Research Services, NIH

LAB/BRANCH
Surgical Neurology Branch

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The angiographic evaluation of experimental cerebral veins occlusion in primates should offer new insight into the general problem of cerebrovascular occlusive disease.

We should be able to establish, first in the animal and eventually in man, how frequently and in what degree cerebral venous occlusion is responsible for the "stroke" syndrome.

Project terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01866-07 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Cerebral Blood Flow by Radiographic and Radioisotopic Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Di Chiro	Head, Section on Neuroradiology &CT	SN NINCDS
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	P. C. Williams	Staff Fellow	SN NINCDS
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	H. Agress, Jr.	Medical Research Analyst	LAS DCRT
	G. S. Johnston	Chief, Nuclear Medicine Dept.	NM CC
	A. E. Jones	Assistant Chief	NM CC
	S. L. Bacharach	Physicist, Applied Physics Section	NM CC
	K. Earle	Chairman, Dept. of Pathology	USUHS

COOPERATING UNITS (if any) Laboratory of Applied Studies, Division of Computer Research and Technology, NIH; Nuclear Medicine Department, CC, NIH; Uniformed Services University of the Health Sciences, Washington, D.C.

LAB/BRANCH
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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	.0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An experimental stroke model in the Rhesus monkey has been developed. The "stroked" animals are being evaluated by x-ray angiography, radionuclide scanning, radionuclide cerebral blood flow determination, radionuclide particles (macroaggregates) transit, Fluorescein angiography, Laser-Doppler velocimetry, microangiography, and autoradiography. The CBF in the experimental infarcted brain is being modified by various physiological, chemical, pharmacological and surgical (revascularization) means. In a collateral experiment various parameters of the cerebral circulation are being studied after production of arterial spasm in the circle of Willis in monkeys. The spasm is caused by total blood - or fractions thereof - introduction within the subarachnoidal space (chiasmatic cistern).

Project Description:

Objectives: Although an enormous number of studies have been carried out on the cerebral blood flow (CBF), the actual behavior of the small cerebral vessels (arteries, capillaries, and veins) in physiologic and pathologic conditions is not yet clear. In fact, in recent years, new concepts--luxury perfusion, intracerebral steal, blue arteries and red veins, loss of autoregulation--have been introduced. These concepts have not brought about increased clarification, but rather additional confusion. We are convinced that morphological analysis of the vessels still remains a potential source of significant information in the area of normal and pathological CBS. We intend, therefore, to pursue the matter of extracting information regarding the circulatory conditions of the brain from radiographic observations, substantiated in selected instances by radioisotopic methods and possibly other diagnostic and research techniques.

Methods Employed: Radiographic and radioisotopic observations of the cerebral circulation represent the main tools to be used in this study.

An experimental stroke model in Rhesus monkeys has been developed. The middle cerebral artery is occluded at its origin through the superior orbital fissure after the removal of the eyeball. This technique (Garcia) offers various advantages. Particularly important is the fact that the produced infarction is consistent in its anatomical location (medial-inferior district of the ipsilateral basal ganglia) and its extent. Also, because no craniectomy is needed, subsequent studies with radioisotopic scanning are easily interpretable.

Two additional technical modalities are used for the evaluation of our "poststroke" experimental animals. One of these modalities is original and consists of the indwelling carotid catheter. This permits us to carry out a variety of injections and blood sample collections at serial intervals during long postoperative periods (weeks and even months). The second modality is the application of standard lucite calvaria for direct observation of the cerebral cortex.

After production of the anemic infarction our multi-pronged evaluation includes:

A) Periodic neurologic testing.

B) Periodic conventional static radionuclide brain scanning.

C) Periodic magnification internal carotid angiography (an indwelling catheter is positioned in the internal carotid artery of some of the stroked monkeys).

D) Periodic radioisotopic angiographic studies (Tc pertechnetate) and periodic ^{133}Xe washout studies (through indwelling internal carotid catheter).

All the radionuclide flow data are gathered with an Anger camera and stored on tape. Subsequently, the data on tape are analyzed by a computer-assisted program. Multiple cursors are drawn in the central part and at the periphery of the infarcted area. The purpose of this approach is to evaluate the centripetal and centrifugal blood flow in the infarcted area at various stages of development of the infarction. The data should be of assistance in the evaluation of the circulation parameters in the initiating, evolving, complete, and resolving stroke. The centripetal and centrifugal flow is evaluated second by second after the intracarotid injection of the radiopharmaceutical.

E) Acute polarographic deep electrodes are used in some of these animals for recording oxygen availability.

F) At various intervals after the creation of the stroke, the animals are sacrificed by a "sudden death" technique and immediately afterwards perfused with a special micropaque solution for post-mortem microangiographic studies.

G) The "sudden death" is accomplished under various attempts at modifying the pathophysiology of the CSF: hyperventilation, hypercarbia, anoxia, hyper-and hypo-tension, use of vasodilators.

H) In a few monkeys perfusion with the radioiodinated albumin macroaggregates for scanning appraisal of the infarcted hemisphere has also been carried out.

I) In selected monkeys the changes of the superficial vasculature are followed up by means of fluorescein angiography of the exposed cortical surface.

J) In selected monkeys Laser-Doppler velocimetry is used to evaluate superficial (cortical) CBF in the various size vessels. This is accomplished with a preparation which includes the lucite calvaria and the indwelling carotid catheter.

K) A day-by-day histological analysis of the anemic infarction is underway. Stroked monkeys are sacrificed for this purpose, one at each sequential day, after the production of the stroke.

L) We plan to study the infarcted monkeys by Computer Assisted Tomography (CAT) as soon as a device for this technique (EMI-Scanner) will be available to us.

M) We are getting ready to start "revascularization" of our infarcted monkeys with an original method of by-pass procedure to the middle cerebral artery distal to the occlusion. The "revascularized" monkeys will be subjected to all the above multi-pronged testing.

In a collateral experiment various parameters of the cerebral circulation are being studied after production of arterial spasm in the circle of Willis in monkeys. The spasm is caused by total blood, or fractions thereof, introduction within the subarachnoid space (chiasmatic cistern).

Major Findings: None

Significance to Bio-Medical Research and the Program of the Institute: We are hopeful that the computer analysis of our radioisotope angiography data in and around the infarcted area will be informative. In particular the determination of the centripetal and centrifugal blood flow in the area of the lesion should enable us to draw some conclusion on the mechanisms of resolution of the infarction and perhaps a better understanding of the important phenomenon of the luxury perfusion.

We are confident that with our stroke model we will be able to contribute both in the basic area of CBF pathophysiology of the infarcted brain, as well as in the practical management of the patients with stroke. Perhaps we will shed some light on such fundamental questions as: a) if and when we should hyperventilate patients with stroke; b) when, if ever, induced hypercarbia may be useful in these patients; c) what is the optimal level at which the blood pressure should be maintained during the various stages of stroke; d) if, when, and which vasodilators should be used.

We have hopes that our "revascularization" approach may prove to be efficacious, practical, and applicable to human patients.

The arterial spasm production after subarachnoidal injection of blood into the chiasmatic cistern is an excellent model simulating the important problem of human arterial spasm following subarachnoidal hemorrhage. This event generally follows rupture of arterial and arteriovenous aneurysms of the brain.

Proposed Course of Project: This is a long-range research project. The evaluation of the cerebral blood flow both in experimental models and in clinical material is still in its infancy, but we are convinced that it will develop into a full discipline.

Publications: Agress, H., Jr., Bacharach, S.L., Hammock, M.K., Green, M.F., Johnston, G.S. and Di Chiro, G.: Stochastic analysis of ^{133}Xe clearance for determining regional cerebral blood flow in a primate model. Stroke 8: 222-226, 1977

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02073-04 SN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Computer Assisted Tomography (Computed Tomography)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	T. Arimitsu	Visiting Scientist	SN NINCDS
	L. Dubal	Visiting Scientist	SN NINCDS
OTHER:	T. N. Chase	Director	IRP NINCDS
	D. B. Calne	Clinical Director	IRP NINCDS
	P. F. Teychenne	Clinical Associate	IRP NINCDS
	A. Neophytides	Clinical Associate	IRP NINCDS
	R. M. Eiben	Acting Chief, Clinical Investigations Service	DMN NINCDS
	C. R. Kollarits	Staff Fellow	CB NEI
	J. L. Doppman	Chief (Cont'd)	DR CC

COOPERATING UNITS (if any) Diagnostic Radiology Dept., CC, NIH; Nuclear Medicine Dept., CC, NIH; Pediatric Oncology Branch, DCT, NCI, NIH; Clinical Branch, NEI, NIH; Dept. of Radiology, GTU Medical School, Washington, DC; Dept. of Neurosurgery, National Naval Medical Center, Bethesda, MD (Cont'd)

LAB/BRANCH
Surgical Neurology Branch

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TOTAL MANYEARS: 2.6	PROFESSIONAL: 2.6	OTHER: .0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Computed tomography (CT) represents the main research area of the Section on Neuroradiology and Computed Tomography. Our experience with a variety of scanners (two head and two body EMI-Scanners at NIH and two ACTA-Scanners at the GTU Medical School) is large and increasing.

Preliminary feasibility tests to build a new type of CT device which will use protons rather than x-rays are under way.

A number of mathematical and physical projects have been completed or are being carried out: simultaneous dual-energy CT using an originally designed split detector; studies of slice geometry in CT; mathematical and physical experimental analysis of a variety of CT artifacts; mathematical improvements of interpolation procedure in CT reconstruction.

Clinical studies include brain, spinal cord and orbit CT research on: normal controls, demyelinating diseases, atrophic processes (including Parkinson and Huntington diseases), postradiation necrosis, oculo-orbital lesions.

Names, Laboratory and Institute Affiliations, etc. (Continued)

OTHER:	J. R. Herdt	Deputy Chief	DR CC
	M. Vermess	Assistant Deputy Chief	DR CC
	G. S. Johnston	Chief	NM CC
	A. E. Jones	Assistant Chief	NM CC
	C. L. Blei	Clinical Associate	NM CC
	D. G. Poplack	Senior Investigator	POB DCT NCI
	N. P. Ramu	Visiting Fellow	POB DCT NCI
	D. Schellinger	Assistant Prof. of Radiology	GTU
	R. A. Pratt	Staff Neurosurgeon	NNMC
	A. L. Martins	Chief, Neurosurgery Service	WRAMC
	A. M. Cormack	Chairman, Physics Dept., Tufts Univ.	
	A. M Koehler	Director, Cyclotron Lab., Harvard Univ.	

Cooperating Units (Continued)

Neurosurgery Service, Walter Reed Army Medical Center, Washington DC; Physics Dept., Tufts University, Medford, MA; Cyclotron Laboratory, Harvard University, Cambridge, MA

Project Description:

Objectives: To advance the clinical applications of CT. Particular attention is being devoted to trying to improve the resolution of the CT devices. Differentiation of the various tissues chemical components through dual-energy scanning or, possibly in the future, by means of proton CT, represent the most promising line of our research. "Tomochemistry" of the CNS is our ultimate goal.

Methods Employed: Clinical CT scanning is now a standard diagnostic procedure. Groups of patients with various disease conditions are studied by CT of the brain and/or body.

A split detector of original design has been built to carry out simultaneous dual-energy scanning.

Basic experiments being carried out as a preliminary step to build the PROTO-Scanner, involve determination in various phantoms of the absorption coefficient of the proton beam produced by a cyclotron. The phantoms include organic materials (particularly organic solutions of various concentrations).

Major Findings: In the clinical area we have:

1) Studied a large number of voluntary normal control patients. Accumulated a large number of cases of demyelinating degenerative processes (leukodystrophies and - in the broadest sense - leukoencephalopathies): in this context we have carried out a study dealing with white-gray matter discrimination with a second generation CT scanner (EMI-CT1010).

2) Studied a large number of Parkinsonians. Many patients affected by Huntington's disease and their next of kin (subjects at risk) have been examined.

3) Observed interesting findings concerning postradiation necrosis of the brain. These findings may mimic brain tumors (recurrence or spread). Their recognition, therefore, is of capital importance.

4) Studied a number of patients with the so-called "atypical" teratoma syndrome. Here we have encountered findings which are quite characteristic, if not pathognomonic (ependymal coating).

5) Analyzed the brain CT findings (demyelination, in particular) after intrathecal methotrexate (administered for controlling meningeal leukemia and CNS metastatic lesions).

6) Continued our work on the spinal cord. We have developed the technique of Computer Assisted Myelography (CAM). This is accomplished after the lumbar injection of metrizamide (a nonionic, hydrosoluble, radiographic contrast medium used in Europe for myelography). We have also continued our research on spinal cord cavities (syringo-hydromyelia). Particularly interesting are some preliminary results on the cord sagittal and coronal images reconstructed from axial transverse scans. A deconvolution program is used for this purpose. Computed tomographic angiography of the spinal cord is described in Project Number Z01 NS 01195-13 SN.

In the physics area the most important findings are:

1) Demonstration of the feasibility of simultaneous dual-energy scanning using an original split detector.

2) The demonstration that protons can be used to detect differences in the physical properties of material at the 0.1% level. This is a significant advance when compared with the ½% resolution limit of the present commercial scanners.

3) Emphasis has been placed on understanding the origin of artifacts which are produced during CT studies of certain areas of the head and the spine.

Significance to Bio-Medical Research and the Program of the Institute: The diagnostic abilities in the area of neuroradiological disease are fundamentally altered by the introduction of CT. The progress in this area is fast. Statements regarding the future significance of this methodology could be surpassed and rendered obsolete in a short time.

Proposed Course of Project: In the Section on Neuroradiology and Computed Tomography, CT will be the main area of research for years to come. We will proceed with a multipronged approach: theory (mathematics, physics); analytical studies; planning and building a new type of CT device; clinical work on the brain, spinal cord and eye; experimental research on primates; tomochemistry of the CNS.

An "International Symposium on Computer Assisted Tomography in Nontumoral Diseases of the Brain, Spinal Cord and Eye," sponsored by NINCDS, took place on the NIH campus, October 11-15, 1976.

The Section is participating in the International Commission on Radiation Units (ICRU) task force on CT definitions.

A new journal "JOURNAL OF COMPUTER ASSISTED TOMOGRAPHY (Computed Tomography)" originates from this Section (Eds. Di Chiro and Brooks).

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Fusner, J.E., Poplack, D.G., Pizzo, P.A. and Di Chiro, G.: Leukoencephalopathy following chemotherapy for rhabdomyosarcoma: Reversibility of cerebral changes demonstrated by computed tomography. J. Pediat. (In press)

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ANNUAL REPORT

July 1, 1976 through September 30, 1977

Laboratory of Central Nervous System Studies

National Institute of Neurological and Communicative Disorders and Stroke

The Laboratory of Central Nervous System Studies comprises two major projects: (1) Neurobiology of Population Isolates--the Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures; and (2) Chronic Central Nervous System Disease Studies--Slow, Latent and Temperate Virus Infections. Both projects are an outgrowth of the Study of Child Growth and Disease Patterns in Primitive Cultures. It was this parent organization that gave rise to the discovery of kuru, a heredofamilial subacute progressive degenerative disease of the central nervous system of the Fore people in the Eastern Highlands of Papua New Guinea and led to the demonstration that kuru is caused by a serially transmissible virus which possesses unconventional biological and biochemical properties. The successful transmission of kuru and the isolation of its virus provided the necessary techniques for the subsequent discovery of a viral etiology for some forms of presenile and senile dementias of man, particularly the Creutzfeldt-Jakob type and more recently certain forms of familial Alzheimer's disease and progressive supranuclear palsy. And, it was this study that has led to the discovery that the agents causing these diseases form a group of transmissible virus-like agents new to the field of microbiology.

Indeed, the most challenging outcome of the discovery that chronic progressive noninflammatory CNS diseases: sporadic, as most cases of Creutzfeldt-Jakob disease (CJD); epidemic, as kuru; or familial, as familial CJD, kuru and some forms of Alzheimer's disease, are "slow infections" caused by viruses with incubation periods measured in years or decades, has been the realization that the etiologic agents of these infections are a new kind of microorganism. Unusual resistance to ultraviolet and ionizing radiation, to formaldehyde, β-propiolactone, and heat, place them in a group unique among viruses. Their ability to produce fatal CNS disease without eliciting inflammatory responses, the failure of the course of disease or incubation period to be influenced by immunosuppression, and failure to demonstrate any antigenicity in high titer infective virus properties, or to find any humoral or delayed hypersensitivity reactions in the diseases, as well as an absence of response to interferon or interference with interferon production, and absence of interference with known viruses, form the series of atypical biological properties which likewise differentiate these agents from any other group of microorganisms. On the other hand, by demonstrating classical virus properties, such as adaptation to new hosts, broadening of host range and reduction of incubation period, dependence on the genetic breed of the host, the presence of strains of differing virulence in wild stock viruses selected by limiting dilution, and interference of fast growing by slow growing strains of scrapie, are all indicative of a complex host-virus genetic interaction characteristic of more classical viruses. Attempts to delineate the chemical nature of the replicating agents, especially to determine whether they are replicated from introduced genetic information or by the induction, derepression or activation of pre-existing genetic information in the host, are the major thrusts of current investigation.

The discovery that the worldwide distributed Creutzfeldt-Jakob disease is caused by a serially transmissible, self-replicating, agent that passes through bacteria, protozoan and fungus retaining membrane filters, the demonstration that the virus is widely distributed in tissues and fluids outside the CNS of affected patients and possesses the physiochemical properties as described above has also resulted in a growing concern among medical and paramedical nursing and laboratory personnel, particularly neurologists, neurosurgeons, pathologists, and anesthesiologists, about the potential hazards involved in caring for patients with presenile dementias and handling their tissues. Concern comes largely from recent reports documenting transmission of Creutzfeldt-Jakob disease by corneal transplant, the accidental inoculation of two patients in neurosurgery with CJD-contaminated electrodes used in stereotactic electroencephalographic recording and stimulation, the suspicion that a neurosurgeon and two general practitioners may have contracted CJD from patients and the characteristic greatly over-represented among patients with CJD of a history of brain or eye surgery in the previous two years before onset of clinical disease. In response to these concerns we have published precautions for conducting biopsies and autopsies and have, more recently, presented a summary on the current knowledge of the pathogenicity and communicability of CJD and related subacute spongiform virus encephalopathies of man and animals which are caused by similar unconventional viruses. We have also made recommendations on the rational precautions that should be taken in caring for these patients and in handling their tissues.

With our demonstration of the transmissibility of scrapie disease from American sheep and English goats to several species of non-human primates, manifested by a disease in the experimental monkey which is indistinguishable from the transmissible virus dementia originating from man, we are confronted with the urgent question of the possible relationship between scrapie of sheep and the spongiform encephalopathies of man. After only one passage through a primate, the scrapie virus was capable of infecting all species of non-human primates tested. However, the Compton (English goat) strain after passage through non-human primates no longer induces disease when inoculated back into sheep, goat or several strains of mice known to be susceptible to scrapie. Thus, the biological properties of scrapie appear to be altered after passage through the primate host-behavior, not unlike classical viruses. If scrapie and the human diseases are caused by similar viruses, such altered biological properties may account for the failure of CJD and kuru viruses to induce disease in mice. We have experienced difficulty in adapting the virus of CJD to mice and guinea pigs, but in recent experiments some passage lines of CJD have caused spongiform encephalopathy in both guinea pigs and mice.

The elucidation of the etiology and epidemiology of a rare, exotic disease restricted to a small population isolate--kuru in New Guinea--has now brought us to worldwide considerations that have importance for all of medicine and microbiology. For neurology, specifically, we have considerable new insights into the whole range of presenile dementias, and in particular, to the larger problems of Alzheimer's disease, familial and senile dementias, and the processes of CNS aging. The implications of vertical transmission of slow virus infections, of conjugal transmission of these diseases, and of host genetic control of disease expression for all genetic diseases, and the relationship of these slow virus infection processes to those which may lead to neoplastic transformation are obvious.

The major problems among the degenerative diseases of multiple sclerosis, amyotrophic lateral sclerosis, and Parkinsonism remain unsolved although there are tantalizing laboratory and epidemiological data pointing to the possible role of virus-like agents in these diseases. Perhaps the masked and defective slow infections with conventional viruses such as are seen in PML and SSPE may provide the best leads for studying these diseases.

The foci of high incidence of amyotrophic lateral sclerosis with associated high incidence of parkinsonism-dementia complex among the Chamorro people on Guam and the Japanese of the Kii Peninsula remain continuing challenges. Our discovery in 1963 and re-evaluation, during the 1974, 1975 and 1976 field trips to the South Pacific, of the very small but very intense foci of such motor neuron diseases with associated high incidence of Parkinsonism, parkinsonism-dementia, and other peculiar bradykinetic and myoclonic dementia syndromes among the Auyu and Jakai people in a remote population of West New Guinea, suggests strongly that some common etiological factor may underlie the occurrence of all these very different syndromes, as they occur strangely in this one small population and are not found in the much larger surrounding populations. Our knowledge of the ALS-PD focus was extended in the 1976 survey to include several additional villages in West Irian with a high incidence of ALS and many ALS deaths, occurring since 1965. A program of demographic and epidemiologic field work to intensively study these foci will be conducted within the coming fiscal year. This will require 4-6 months of intensive field study. This year we have intensified our efforts, both in the field as well as the laboratory, to further delineate the ALS-PD complex on Guam. The program has been expanded to include virological, genetic, epidemiological, immune function, radiographic, demographic, anthropological, morphometric, therapeutic, and pathological studies of these two neurological disorders. In addition, all original registries have been updated and much of the new data is already under analysis. We are also conducting a wide survey of ALS and all other chronic disabilities in the large Chamorro population who have immigrated from Guam to the U.S.A., in whom the high incidence of ALS or PD continues to appear.

In other areas of Micronesia human biological field and laboratory studies continue. Studies of asthma and other chronic respiratory diseases indicate that 75% of the children under 5 years of age were found to have asthma while over 50% of the adults over age 40 were affected by chronic bronchitis often with an asthmatic component, and typical chronic obstructic airway disease occurred in almost one-third of the male population over 50 years of age. As a result, pulmonary airway diseases constitute the most important cause of morbidity and mortality in the Western Caroline Islands.

The models of lysogenicity and of subviral genetically active macromolecular structures from the study of bacterial viruses and bacterial genetics supply ample imaginative framework for an expression of our ideas of possible mechanisms of infectious pathogenesis in man. The unconventional viruses of the spongiform encephalopathies tax even our imagination in relation to molecular biology gained from these studies in bacteria.

For a now-disappearing disease, kuru, in a small primitive population to have brought us this far is ample reason for pursuing intensively the challenges offered by the still inexplicable high incidence and peculiar profusion of different neurological syndromes, pathologically distinct yet apparently somehow related to each other, which have been discovered in the several small population enclaves we have investigated. Thus, the high incidence of ALS, ALS/PD on Guam and among a small population of people in West New Guinea, coupled with the high incidence of ALS on the Kii peninsula of Japan may indeed offer the best opportunity of solving the problem of this sclerosing disease which in the United States has an incidence as high as that of multiple sclerosis.

The delineation of infection as the etiology of heredofamilial and presenile and senile dementias of man was made possible only through the concomitant studies of the neurobiology of population isolates. In this area we have been engrossed in the investigation of deaf-mutism, mental subnormality and other congenital central nervous system defects associated with endemic goiter in the Central Highlands of Western New Guinea, as well as patterns of delayed puberty, slow growth rates, and of early aging in isolated Melanesian groups. Ethnic drug abuse (particularly of kava), strange patterns of psychosexual development, pseudohermaphroditism, and culturally-determined responses to pain, and roots of aesthetic expression, have all been under study. Foci in primitive population isolates of familial periodic paralysis, progressive muscular dystrophy (both the pseudohypertrophic type of Duchenne and the non-pseudohypertrophic distal type), amyotrophic lateral sclerosis and Parkinsonism, are also being investigated. Genetic studies on human evolution led to the discovery of new genetic factors among haptoglobin, hemoglobin, and red cell enzyme pheomorphisms and the definition of their biochemical structure.

The further significance of scientific investigations of small population enclaves of remote populations has been even more dramatically apparent during the 1975-1976 field trip with our re-evaluation of what may turn out to be done of the largest "epidemics of epilepsy" ever recorded. This is occurring in the Wissel Lakes area of West New Guinea and is the result of cysticercosis, with the larvae of Taenia solium, the pig-tape worm, which has been newly introduced into New Guinea. Our recent studies have led us to conclude that the natural history of cysticercosis epilepsy is not a result of death of the worm, scarring and calcification of lesions, as much of the literature suggests, but is an early sign of inflammation from new invasion of the brain by the Taenia larvae. First, convulsions often occur even before the first subcutaneous nodules appear, and as the nodules increase in number, additional seizures occur. The high incidence of severe third degree burns, which may even result in death, is a direct result of cysticercosis-induced unexpected seizures which occur during the sleeping hours, throwing the patient into the house fire. The unclothed people, living at 2000 meter elevation, need to sleep close to the home fires on cold nights. We are able to date the first introduction of Taenia solium into the area and to plot the spread of

Taeniasis in pigs and man, and of cysticercosis and associated epilepsy in man to other previously Taenia-free areas. With Dr. Budi Subianto, the local Indonesian Medical Officer, we have planned a neuroepidemiologic study aimed at elucidating the natural history of the epilepsy and acute psychoses and other neurological complications which have occurred concomitantly with the emergence of subcutaneous cysticercosis nodules.

Dr. Gajdusek has been invited by Soviet investigators as the consultant to their study of the huge focus of a chronic degenerative basal ganglia extrapyramidal disorder called Vilyuisk encephalitis, which is restricted to the Yakut people in eastern Siberia. For his visit, many investigators, including the Minister of Health of the Yakut Republic, were brought to Moscow, along with four patients suffering from the advanced stages of the disease. There were three days of presentations of the field epidemiological, clinical and laboratory data which had been accumulated, and several hours of discussions on the direction the future studies should take. The disease is a syndrome which has not been previously encountered elsewhere. There is a high possibility that it is a slow virus infection. Our Soviet colleagues have indicated that they will invite us to participate in future meetings devoted to this disease.

The development and maturation of the two major projects of this laboratory resulted from cross-fertilization of each since their origin, and both have grown from the basic studies on child growth and development and disease patterns in primitive cultures. And, although the two projects, each composed of many subsections, differ markedly in the inquiries they phrase and the techniques of investigation they employ, much of the field data collected from one project is also requisite for the studies in other projects. Both are served by the same investigators who function as a team. These scientists derive their creative stimulus, dedication and enthusiasm, to a great extent from the atypical and exotic biological, social, and cultural materials presented and the diverse, frequently unconventional approaches phrased by the two projects.

Principal Investigators: D. Carleton Gajdusek, M.D.
Clarence J. Gibbs, Jr., Ph.D.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01282-13 CNSS

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PRINCIPAL INVESTIGATORS: D. Carleton Gajdusek, M.D., Chief, LCNSS; Clarence J. Gibbs, Jr., Ph.D., Assistant Chief, LCNSS; David M. Asher, M.D., Paul W. Brown, M.D., and Ralph Garruto, Ph.D.

Michael Alpers, M.D.; Richard Benfante, M.A.; Peter Fetchko, M.A.; Josede Figirliyong, M.A.; Paul M. Hoffman, M.D.; Chev Kidson, M.D.; Robert L. Kirk, Ph.D.; Klaus Mannweiler, M.D.; Colin Masters, M.D.; Ivan Mbagintao; Judith Meyer; Steven Ono; Robert Rohwer, Ph.D.; Donald Rubinstein; Roger Traub, M.D.; Vincent Zigas, M.D.

Jacques Bert, M.D.; Francoise Cathala, M.D., Louis Court, M.D.; Arwin R. Diwan, Ph.D.; Richard Feinberg, Ph.D.; Father David Galles; Undapmaika Kalagune; Robert MacLennan, M.D.; Jesus Raglmar; Wulf Schiefenhovel, M.D.; Koiye Tasa

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LAB/BRANCH
Laboratory of Central Nervous System Studies, Intramural Research Program

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	8	4

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Studies of human biology of vanishing primitive societies focus on neurological development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Laboratory techniques of molecular biology,immunology,virology,endocrinology and biochemistry in these cultures and field epidemiological,genetic and clinical studies are aimed at problems more appropriately studied in small isolated primitive bands than in civilized societies. Data and specimens collected over years on expeditions to Micronesia,Polynesia,Solomon Islands,New Hebrides,New Guinea, Indonesia,S.America,Asia and Africa are used. Studies on nutrition,reproduction, fertility,neuroendocrine influences on age of sexual maturation and aging,genetic polymorphisms,genetic distance,unusual and odd employment of the higher cerebral CNS function of language learning,cognitive styles,computation(calculation without words or numbers),and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we would be unable to investigate once the natural cultural experiments in primitive human isolates were amalgamated into the cosmopolitan community of man. Foci of high incidence prevalence kuru, ALS/PD,epilepsy,other neurological degenerations,hysterical disorders,schizophrenia, neoplasms,goiter,cretinism,rheumatoid diseases,diabetes,goit,asthma,chronic lung disease,malaria,filariasis,leprosy,cysticercosis and other infections are investigated.

COOPERATING UNITS: continued

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ITALY: M. and Dr. A. Jablonko, Perugia.

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COOPERATING UNITS: continued

Dr. G. Gibson, Smithsonian Institution; Hawaii--Dr. A. Diwan, University of Hawaii School of Medicine, Honolulu; Drs. L. Rosen, G. Wallace and R. Tesh, Pacific Research Station, NIAID, Honolulu; Maryland--Drs. K. Shah, R. Roos, Department of Neurology, Johns Hopkins University Hospital, Baltimore; Dr. K. Brown, Dr. W.C. Leyshon, Laboratory of Developmental Biology and Anomalies, NIDR, NIH, Bethesda; Dr. P. MacLean, Laboratory of Brain Evolution and Behavior, NIMH, NIH, Bethesda; Drs. F. Neva, L.H. Miller, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda; Dr. J. Wolff, Clinical Endocrinology Branch, NIAMH, NIH, Bethesda; Dr. J. Sever, Dr. S. Houff, NINCDS, NIH, Bethesda; Dr. C. Wisseman, School of Medicine, University of Maryland, Baltimore; Dr. T.C. Raines, National Bureau of Standards, Gaithersburg; Massachusetts--Dr. P. Fetchko, E. Dodge, Peabody Museum, Salem; Dr. N. Geschwind, Neurology Unit, Beth Israel Hospital, Boston; Dr. John Enders, Dr. M. Oxman, Dr. R. Ferber, Children's Hospital Medical Center, Boston; L.K. Marshall, Boston; K. Muller, Harvard University, Cambridge; Michigan--Dr. E.A. Rodin, Department of Mental Health, Lafayette Clinic, Detroit; Dr. T.M. Ernst, Department of Anthropology, University of Michigan, Ann Arbor; New York--Dr. Rogert Traub, I.B.M., Yorktown Heights; Dr. R.E. Peterson, Department of Medicine, Cornell Medical Center, New York; Dr. P. Kennedy, Program of American Studies, State University of New York, Buffalo; Dr. R. Glasse, Queens College, Flushing; Dr. S. Lindenbaum, York College, CUNY, Jamaica; Dr. Alan Lomax, Applied Social Research, Columbia University, New York; Dr. Margaret Mead, American Museum of Natural History, New York; E.L. Schiefflin, Fordham University, Bronx; Ohio--Dr. A. Steinberg, Case Western Reserve University, Cleveland; Pennsylvania--Dr. D. O'Brien, Department of Anthropology, Temple University, Philadelphia; Dr. N. Chagnon, Dr. P.T. Baker, Pennsylvania State University, University Park; Rhode Island--Dr. T. Kiefer, Brown University, Providence; Washington--Dr. R. DiGiacomo, Department of Epidemiology, Dr. P. Kunstadter, Department of Preventive Medicine, University of Washington, Seattle.

VENEZUELA: L.T. Laffer and F. Melchiorri, Caracas.

Sub-Project I: Study of the developmental patterning of the human nervous system (cybernetics of human development).

Sub-Project II: Human evolutionary studies in isolated primitive groups.

Sub-Project III: Studies of isolated Micronesian populations.

Sub-Project IV: Studies of isolated New Guinea populations.

Sub-Project V: Studies of Australian Aborigines.

Sub-Project VI: Studies of isolated New Hebrides and Solomon Islands populations.

Sub-Project VII: Studies of Central and South American Indians.

Sub-Project VIII: Developmental, genetic and disease patterns in primitive populations of Asia, Africa, Indonesia, Melanesia, Micronesia, Polynesia and the Arctic.

Sub-Project IX: Experimental developmental neuropediatrics in infantile programming: an empirical approach to the language of information input into the nervous system.

Sub-Project X: Ciphers and notation for the coding of sensory data for neurological information processing.

Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.

Sub-Project XII: Studies of high incidence of neurological diseases in specific racial and ethnic groups and in primitive or geographic population isolates.

Project Description: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures (described fully on pages

Publications: Listed on pages 20r to 25r.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 00969-13 CNSS

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PRINCIPAL INVESTIGATORS: D. Carleton Gajdusek, M.D., Chief, LCNSS; and Clarence J. Gibbs, Jr., Ph.D., Assistant Chief, LCNSS

Herbert L. Amyx, D.V.M.; David M. Asher, M.D.; Richard Benfante, M.A.; Paul W. Brown, M.D., Hiroshi Oda, M.D.; Ralph Garruto, Ph.D.; Colin Masters, M.D.; George J. Nemo, Ph.D.; Robert Rohwer, Ph.D.; Lon R. White, M.D.

Michael Alpers, M.D.; Jacques Bert, M.D.; Francoise Cathala, M.D.; Huyn J. Cho, D.C.M., Ph.D.; Louis Court, M.D.; Arwin R. Diwan, Ph.D.; Lydia Fadeeva, M.D.; Chev Kidson, M.D.; Klaus Mannweiler, M.D.; Marie-Claude Moreau, Ph.D.; Ryoichi Mori, M.D., Ph.D.; Maria-Teresa Borras Puga; Peter Rytik, M.D.; Volker ter Meulen, M.D.; Roger D. Traub, M.D.

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LAB/BRANCH
Laboratory of Central Nervous System Studies, Intramural Research Program

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
24	14	10

CHECK APPROPRIATE BOX(ES)

XX(a) HUMAN SUBJECTS   XX(b) HUMAN TISSUES   ☐ (c) NEITHER

XX (a1) MINORS   ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, parkinsonism-dementia, Parkinson's, Pick's and Alzheimer's diseases, Huntington's chorea, supranuclear palsy, other presenile dementias, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, SSPE, PML, dialysis encephalopathy, and intracranial neoplasms. Even familial, apparently hereditary diseases may be slow virus infections. Subacute spongiform virus encephalopathies (kuru and Creutzfeldt-Jakob (CJD) diseases of man; scrapie and mink encephalopathy) are caused by unconventional viruses with unique properties posing important theoretical problems to microbiology and molecular biology; a major goal is elucidation of their structure and mechanisms of replication. Transmissible virus dementias are increasingly recognized worldwide causes of death: high incidence foci, transmission by corneal transplant or brain surgery, and occupational hazards from exposure to brain occur. In order to determine the usual mode of infection with the virus, a worldwide epidemiological study of transmissible virus dementia (CJD) dases is underway with special attention to familial clusters of cases and with a quest for possible relationship of scrapie of sheep to the human disease.

COOPERATING UNITS: continued

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Sub-Project I: Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible heredofamilial diseases, presenile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.

Sub-Project II: Characterization and pathogenesis of kuru virus.

Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia) virus.

Sub-Project IV: Scrapie: studies on the purification, physical and biological characterization and nature of the virus.

Sub-Project V: *In vivo* and *in vitro* host range of the subacute spongiform virus encephalopathies.

Sub-Project VI: Isolation and characterization of adenovirus from the urine of chimpanzees.

Sub-Project VII: Fluorescent antibody studies on the intracellular localization and identification of viral antigens *in vivo* and *in vitro* in tissues from patients with subacute diseases of the central nervous system.

Sub-Project VIII: Tissue and cell culture *in vitro* studies of virus induced slow infections of man and animals.

Sub-Project IX: The syncytium forming viruses (simian and human foamy viruses).

Sub-Project X: Studies on transformed human brain tissue *in vitro* and characterization of associated virus.

Sub-Project XI: Electron microscopic membrane studies of subacute spongiform virus encephalopathies.

Sub-Project XII: Characterization and identification of new herpes viruses from explant cultures of tissues from subhuman primates.

Sub-Project XIII: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.

Sub-Project XIV: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.

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Sub-Project XV: Development of serological and immunological test system for use in the study of slow infections of the central nervous system.

Sub-Project XVI: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remission and exacerbation.

Sub-Project XVII: Animal management and intercurrent diseases in subhuman primates on long-term studies of slow infections.

Sub-Project XVIII: Studies to determine the possible presence of cryptic viral genomes in human brain tissues.

Sub-Project XIX: Sequential development of kuru induced neuropathological lesions in spider monkeys.

Sub-Project XX: Studies on the isolation, characterization, identification and pathogenicity of type C viruses from human and animal tissues.

Sub-Project XXI: Biochemical studies of the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia.

Sub-Project XXII: *In vitro* transformation of CNS cells persistently infected with subacute spongiform virus encephalopathies.

Sub-Project XXIII: Study of mitochondrial mutants from scrapie-infected mouse brain cells.

Previous Serial Number: Z01 NS 00969-12 LCNSS

Project Description: Chronic Central Nervous System Disease Studies (described fully on pages

Publications: Listed on pages 20r to 25r.

The projects (I through XXIII) listed herein, as itemized in the Project Reports of previous years, have continued throughout this year and have been expanded, as are reflected in the extensive list of publications and the summary in pages 20r-25r . Contractural phases of this work are being conducted at:

Gulf South Research Institute, New Iberia, Louisiana
Public Health Research Institute of New York, Otisville
Center for Primate Biology, University of California, Davis
Department of Molecular Biology, University of Connecticut

Publications

Asher, D.M., Gibbs, C.J. Jr., and Gajdusek, D.C. Pathogenesis of subacute spongiform encephalopathies. Annals of Clinical and Laboratory Science 6:1, 84-103, 1976.

Asher, D.M., Yanagihara, R.T., Gajdusek, D.C., and Gibbs, C.J. Jr. Search for transmissible agents in pediatric patients with neurological diseases of unknown etiology. Abstract No. 869 in Abstracts of the Forty-sixth Annual Meeting of the Society for Pediatric Research, St. Louis, April 28-30. Pediatric Research 10:4 (April), 446, 1976.

Brody, J.A., and Gibbs, C.J. Jr. Chronic neurological disease. Subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, kuru, Creutzfeldt-Jakob disease. Chapter 24 in: "Viral Infections of Man", A.S. Evans, editor. Plenum Press, New York, pp. 519-537, 1976.

Brown, P., Collins, W.E., Gajdusek, D.C., and Miller, L.H. An evaluation of malaria fluorescent antibody patterns in several remote island populations of the New Hebrides, Solomons, Western Carolines, and New Guinea. American Journal of Tropical Medicine and Hygiene 25:6, 775-783, 1976.

Brown, P., and Morris, J.A. Serological response to BK virus following human infection with SV40. Proceedings of the Society for Experimental Biology and Medicine 152:1 (May), 130-131, 1976.

Chou, S.M., Payne, W.N., Gibbs, C.J. Jr., and Gajdusek, D.C. Transmission and scanning electron microscopy on status spongiosus in Creutzfeldt-Jakob disease. Abstract No. 35 in Abstracts of the Fifty-second Annual Meeting of the American Association of Neuropathologists, San Francisco, June 11-13, p. 317, 1976.

Espana, C., Gajdusek, D.C., Gibbs, C.J. Jr., and Lock, K. Transmission of Creutzfeldt-Jakob disease to the patas monkey (*Erythrocebus patas*) with cytopathological changes in *in vitro* brain cells. Intervirology 6: 150-151, 1975/76.

Gajdusek, D.C. Conjectural natural history of kuru and Creutzfeldt-Jakob disease based on recent epidemiologic and virologic data on the subacute spongiform virus encephalopathies. Presented at the Fourth Pan American Congress of Neurology, Mexico City, October 12-17, 1975. Abstract in Current Reports in Neurology 2:1, 19, 1976.

Gajdusek, D.C. Infectious agents in rheumatic disease. In: "Arthritis: Out of the Maze", Volume 2. DHEW Publication No. (NIH) 76-1151, National Institutes of Health, Bethesda, Maryland (April), pp. 285-288, 1976. Also in: Report of the Research Work Group of the National Commission on Arthritis and Related Musculoskeletal Diseases. National Institutes of Health, Bethesda, Maryland (November), pp. 114-127, 1975.

Gajdusek, D.C. *Pneumocystis carinii* as the cause of human disease: historical perspective and magnitude of the problem. Introductory remarks. In: Symposium on *Pneumocystis carinii* Infection, J.B. Robbins, V.T. DeVita, Jr., and W. Dutz, editors. Monograph 43, National Cancer Institute, Bethesda, Maryland (October), pp. 1-11, 1976.

Gajdusek, D.C., Alpers, M.P., and Gibbs, C.J. Jr. Kuru: epidemiological and virological studies of a unique New Guinean disease with wide significance to general medicine. In "Essays on Kuru", R.W. Hornabrook, editor. Monograph No. 3, Institute of Human Biology, Goroka, Papua New Guinea, 1976.

Gajdusek, D.C., Gibbs, C.J. Jr., Collins, G., Traub, R.D. Survival of Creutzfeldt-Jakob disease virus in formol-fixed brain tissue. New England Journal of Medicine 294:10 (March 4), 553, 1976.

Garruto, R.M. Hematology. Chapter 15 in: "Man in the Andes: A Multidisciplinary Study of High-Altitude Quechua", P.T. Baker and M.A. Little, editors. U.S. International Biological Programme Synthesis Vol. 1, Dowden, Hutchinson and Ross, Stroudesburg, Pennsylvania, pp. 261-285, 1976.

Garruto, R.M., and Hoff, C. Genetic history and affinities. Chapter 5 in: "Man in the Andes: A Multidisciplinary Study of High-Altitude Quechua", P.T. Baker and M.A. Little, editors. U.S. International Biological Programme Synthesis Vol. 1, Dowden, Hutchinson and Ross, Stroudesburg, Pennsylvania, pp. 98-117, 1976.

Gibbs, C.J. Jr., and Gajdusek, D.C. Studies on the viruses of subacute spongiform encephalopathies using primates, their only available indicator. In: First Interamerican Conference on Conservation and Utilization of American Nonhuman Primates in Biomedical Research, Lima, Peru, June 2-4, 1975. Scientific Publication No. 317, Pan American Health Organization, Washington, D.C., pp. 83-109, 1976.

Lewis, M.A., Frye, L.D., Gibbs, C.J. Jr., Chou, S.M., Cutchins, E.C., Gajdusek, D.C., and Ward, G. Isolation and characterization of two new herpes-like viruses from capuchin monkeys. Infection and Immunity 14:3 (September) 759-766. 1976.

Masters, C.L., Alpers, M.P., Gajdusek, D.C., Gibbs, C.J. Jr., and Kakulas, B.A. Experimental kuru in the gibbon and sooty mangabey and Creutzfeldt-Jakob disease in the pigtailed macaque. Journal of Medical Primatology 5:4, 205-209, 1976.

Masters, C.L., Kakulas, B.A., Alpers, M., Gajdusek, D.C., and Gibbs, C.J. Jr. Preclinical lesions and their progression in the experimental spongiform encephalopathies (kuru and Creutzfeldt-Jakob disease) in primates. Journal of Neuropathology and Experimental Neurology 35:1 (November-December), 593-605, 1976.

Mbaginta'o, I. Medicine practices and funeral ceremony of the Dunkwi Anga. Journal de la Societe des Oceanistes 32:53 (December), 299-305, 1976.

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Siakotos, A.N., Bucana, C., Gajdusek, D.C., Gibbs, C.J. Jr., and Traub, R.D. Partial purification of the scrapie agent from mouse brain by pressure disruption and zonal centrifugation in sucrose-sodium chloride gradient. Virology 70:1 (March), 230-237, 1976.

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ANNUAL REPORT

July 1, 1976 through September 30, 1977
Clinical Neurosciences Branch
National Institute of Neurological and Communicative
Disorders and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

As in the past, the activity of this Branch can be divided into research and clinical-diagnostic service, the latter involving a total of 3.9 man/years (0.8 professional and 3.1 technical-clerical).

1. Clinical-Diagnostic Service

From the time the last Report was prepared (April 12, 1976) to that of the present Report (May 31, 1977) a total of 1420 EEG tracings were obtained and interpreted, in patients referred to our Branch as part of their routine clinical investigation or for specific research projects originating from other Branches and/or Institutes. This represents about 110 examinations per month, and the distribution of these referrals according to the various Institutes of origin has been:

INSTITUTE	NO.	%
NINCDS	773	54.5
(OPD)	(235)	(16.5)
NIMH	205	14.5
NCI	203	14.3
NIAMD	76	5.3
NCHD	68	4.8
NHLI	56	3.9
NIAID	32	2.2
Misc	7	0.5
TOTAL	1420	100.0

In comparison with the past activity of this Service, the total number of referrals remains rather stationary but one notes a slight decrease of referrals from NINCDS and a relative increase of examinations requested by other Institutes. A fair number of these 647 requests by Institutes other than NINCDS had to be carried out on the ward, at the patient's bed or in the Intensive Care Unit. As already mentioned in the previous Report, the reduction in technical staff (2 EEG technologists versus 3-4 in the past) has occasionally interfered with the handling of emergency referrals and the service has unavoidably suffered during the annual and/or sick leave of the technicians. The service has provided in the past and continues to provide useful material for several investigation projects in our Branch as well as for the training in Clinical Electroencephalography which benefits one or two clinical associates every year.

2. Research Activity

Of the research projects mentioned in the previous Report, six had been completed and two were terminated. The present Report includes a total of 15 projects of which three are new and the others represent a continuation (both active and inactive) of projects initiated in the past.

a) Clinical

Several of the clinical projects originating from the neurosurgical unit of the Section of Functional Neurosurgery have remained at the level of the planning stage due to the still unsettled situation in that Section, the limited allocation in the number of beds and the consequent difficulty in recruiting a clinical Associate. The latter problem is apparently close to a satisfactory solution and this should make it possible to initiate at least some of these projects toward the end of the current fiscal period.

As part of a major, long standing, project on epileptogenic mechanisms in man, a detailed study was recently completed on the results of chronic cerebellar stimulation in patients with intractable seizures. This study, which consists of a 20-24 month follow-up, has failed to show any significant improvement in seizure frequency following the cerebellar electrode implantation and during the procedure, or any relationship between seizure frequency and periods of cerebellar stimulation or non-stimulation. This objective, negative findings were in marked contrast with the subjective highly favorable reactions of the patients and the satisfaction of their families for the procedure. The CSF concentration of norepinephrine increased and that of GABA decreased following cerebellar stimulation. It seems possible that the elevation of mood, as a consequence of a NE rise in CNS, be the most important result of the stimulation procedure.

Another project dealing with the clinical features and ictal patterns in epileptic patients with electrographic evidence of temporal lobe involvement has been completed. This study is based on the historical data and subjective ictal phenomena reported by 270 such patients and on the ictal manifestations observed in the course of at least six seizures in each of 199 of these patients. The findings are summarized in the individual project description and are about to appear in print. It is generally believed that, temporal lobe seizures mainly consist of automatic behavior. On the basis of this study, however, it would appear that partial motor phenomena of a tonic or clonic type also play an important role in such seizures. On the other hand, automatisms are rather commonly observed in seizures which are apparently originating from other cortical regions and only those consisting of highly complex, purposeful and well organized activity are suggestively pathognomonic of a primary involvement of temporal lobe structures. The data also fail to support the concept of an association between "temporal lobe epilepsy" and violence. These and other findings, complementing those of previous similar studies in other groups of epileptic patients with partial seizures, should prove of practical usefulness in the localizing diagnosis and classification of seizure disorders.

A project started in the current fiscal period deals with a study of the clinical manifestations in patients with seizure disorders and EEG abnormalities localized over the midline, central vertex region. This study is still in the stage of data collection and should be completed in the next fiscal period.

The purpose of another new project is to assess the usefulness of visual responses in the diagnostic evaluation of neurologic and neurophthalmologic disorders. Photic stimulation is routinely performed in all suitable cases at the end of their routine EEG examination and the topography, morphology, latency and amplitude of the response are evaluated. No major findings are yet available but the project should be completed within the next fiscal period.

There are three ongoing projects from the neuropsychology unit of the Section of Functional Neurosurgery. One attempts to identify brain mechanisms which regulate perception and the storage and retrieval of information as well as to identify the significance of brain dysfunction in psychiatric patients by means of electrophysiological techniques. This study is still in progress and the data are currently being evaluated. Another project has the dual purpose of: a) identifying personality profiles of epileptics with confirmed temporal lobe seizures in comparison with two matched control groups and b) evaluating sensory disturbances in olfactory and gustatory modalities in the same type of patients. On the basis of preliminary results, the temporal epileptic group appears to have an abnormal personality profile and also to demonstrate a higher degree of unusual behavior. This seems to be related to the duration of the seizure disorder rather than to the frequency of seizures. Furthermore patients with temporal lobe epilepsy, while capable of detecting the presence or absence of each taste and smell stimuli, had serious difficulty in identifying them; a finding that confirms the important perceptual role of the temporal lobe. The objectives of the third project are to: a) assess the effects of localized brain injuries on language functions and memory; b) compare such effects to those due to congenital or developmental conditions and c) examine the role of the temporal lobe in guiding visual behavior under distorted conditions. Part of this project is carried out in collaboration with the University of Washington in Seattle. Major findings recently obtained in relation to this study are outlined in detail in the project description and in two publications, but the project itself is expected to continue through the coming fiscal period.

The identification of individuals at risk for Huntington's disease is the object of another study. This is carried out by means of a battery of auditory, visual and tactile tasks presented to selected subjects to evaluate their perception, memory and visuomotor and eye-hand coordination. The same subjects are also evaluated from a neuropsychiatric viewpoint (MMPI) and tested for somatic preoccupation, anxiety and depression. This study, which is part of a major, longstanding project on involuntary movements, is still in the process of data collection and is expected to continue through the coming fiscal period.

b) Experimental Research

One of the main interests of the Branch has always been the pathophysiology of epilepsy and, in particular, the investigation of the cellular mechanisms which are at the basis of epileptiform activity. This interest is reflected in several of the experimental projects. One of these, initiated in the previous fiscal period, has been recently completed and two papers are about to be submitted for publication. This study utilizes the experimental model of acute, partial cortical epilepsy in cat with local penicillin application and was designed to determine whether amino acid antagonism or facilitation is involved in the epileptogenic action of this substance. The study involves a sophisticated technique for both the extracellular recording of cortical unit activity and the simultaneous microiontophoretic application of various amino acids and of different epileptogenic agents. In contrast with the iontophoretic application of other convulsant drugs, such as bicuculline and strychnine which show a specific antagonistic action, respectively versus GABA and Glycine, penicillin iontophoresis reversibly but nonspecifically antagonized the three inhibitory amino acids tested (Glycine, alanine and GABA). This antagonism clearly manifested itself with low iontophoretic current (100-500 nA) for penicillin, prior to the onset of the typical epileptiform manifestations induced by the substance. Also, no modification of endogenous inhibition by penicillin iontophoresis could be demonstrated. On the basis of these and other findings it would appear that the primary mechanism for this type of epileptogenesis does not depend on an alteration of specific amino acid responses, at least of the three potential neurotransmitters which were tested.

One of the most characteristic electrographic expressions of epileptiform manifestations is neuronal hyperactivity and this is also observed in a number of CNS structures following deafferentation. Indeed, it has been suggested that some form of deafferentation-induced hyperactivity plays an important role in various types of epileptogenic lesions of the brain. Such type of hyperactivity is generally interpreted to reflect post-synaptic hypersensitivity to excitatory neurotransmitters although it has also been suggested that it may result from postsynaptic membrane damage due to transneuronal atrophy. Another project was designed to determine which of these two mechanisms (denervation sensitivity or transneuronal atrophy) is responsible for deafferentation hyperactivity, and this study has been carried out in the trigeminal nucleus of cat, following retrogasserian trigeminal rhizotomy. Six-barrelled micropipettes were used to record single unit activity in this nucleus with simultaneous iontophoresis of potential amino acids neurotransmitters. Whereas all neurons in the control (intact) trigeminal nucleus were consistently inhibited by GABA, alanine and glycine and excited by homocysteic acid, glutamate and aspartic acid, the deafferented neurons exhibited either a normal response to such amino acids or a markedly decreased response to both inhibitory and excitatory amino acids. These and other results appear to be inconsistent with Cannon's law of denervation supersensitivity. Rather, deafferentation hyperactivity and amino acid hyposensitivity would more likely suggest, transneuronal atrophy. This project is about to be completed and a paper is in preparation.

Continuing the series of studies initiated in our Laboratory several years ago on the cortical metabolic concomitants of seizure activity, the NADH fluorescence method has been re-evaluated in its validity when applied to relatively large areas of the cortex. This study is the continuation of a project originally initiated in the previous fiscal period (by different investigators and with a somewhat different title). By using different references and different methods of calibration, results have been obtained which seem to suggest that the measurement of NADH fluorescence may be seriously contaminated by changes in hemoglobin concentration in the field. Thus, the current methods, when applied to large areas of the cortex, are probably sensitive to both changes in blood flow and in metabolic activity. This conclusion is apparently confirmed by another recently initiated study. This was aimed at correlating the electrocorticographic DC changes which are observed during the development of epileptiform activity with the changes in cortical fluorescence both at the center of a focus and at various distances, in the tissue surrounding it. Indeed, whereas onset and peak of the paroxysmal DC shifts showed a progressive delay as the recording electrode was moved away from the center of the focus, cortical fluorescence changes during ictal episodes were rather variable and could not be consistently correlated with either the distance from the center of the focus or the negative DC shift. Actually, it was not unusual to find a decrease in cortical fluorescence of much greater amplitude when this was measured in close proximity to a large blood vessel than in a cortical region deprived of major vessels. These observations would indicate that at least part of the fluorescence change, commonly interpreted as reflecting oxidation of NADH may be in reality due to changes in blood flow, associated with local epileptic activation.

Another project deals with maturational changes in the neuronal-glial reaction of the hypoglossal nucleus to axonal injury. This work was carried out in rats, 7, 10 and 21 days old. One of their hypoglossal nerves was either crushed or ligated and the two nuclei (intact and operated side) were compared after various periods of survival. The results indicate a differing reaction with age, microglia proliferating quickly in the mature animal while being significantly delayed in the immature preparation. The study will be continued to investigate fate and time course of cytoplasmatic protrusions of neurons observed in immature animals and to apply freeze-etch techniques in the study of these reactive changes.

Two additional studies are reported under another longstanding project. One, deals with the correlation of the action potential and the morphology of regenerating nerve. The other is a study of the experimental meningocerebral cicatrix, including that secondary to operative exposure and the possible effects of ultraviolet irradiation (355 nm) at various energy levels. Both studies are still in the data collection stage but their completion is expected in the next fiscal period.

Other Activities, Honors etc.

The Chief of the Branch is officially and actively involved in editorial duties of various specialized Journals (Electroenceph. clin. Neurophysiol.; Epilepsia; Arch. ital. Sci. Biol.) and is co-editor of the Handbook of EEG and Clinical Neurophysiology. As Past President and member of the Executive Committee of the International Federation of Societies for EEG and Clinical Neurophysiology, he is also actively involved in the organization of the Scientific program of the IX International Congress of said Federation, to be held in Amsterdam, September 1977. He has also been officially invited to participate in one Symposium of the same Congress. He has been elected Foreign Corresponding Member of the Italian Society of Neurology and has been one the invited speakers at the inaugural meeting of the Canadian League Against Epilepsy in Vancouver and at the meeting of the European Congress of Clinical Neurophysiology in Venice. He was also invited to present the first J.K. Merlis Lecture in April 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00100-24 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Epileptogenic Mechanisms in the Brain of Man and Other Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: J. M. Van Buren, M.D.	Associate Chief	CN NINCDS
C. Ajmone Marsan, M.D.	Chief	CN NINCDS
C. R. Lake, M.D.	Clinical Associate	LCS NIMH

COOPERATING UNITS (if any)
Laboratory of Neural Control, NINCDS
Medical Neurology Branch, NINCDS
Laboratory of Clinical Science, NIMH
Division of Endocrinology, National Naval Medical Center

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery
Clinical Neurophysiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.8	0.2

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A detailed study of the results of chronic cerebellar stimulation in 5 patients subject to severe intractable seizures of multifocal nature has been finished with a 20 - 24 mon. follow-up. Despite unanimous approval of the procedure by the patients and their families, regular documentation of the seizure frequency on the ward failed to show any improvement in the seizure frequency over the postoperative period or any relationship between the periods without and with cerebellar stimulation which was applied both in the unblinded and double blind modes. However, studies of the CSF concentrations of both norepinephrine and GABA showed changes related to the stimulation with a rise of the former and a fall of the latter (GABA). Although both of these substances have some suppressive effect upon seizure states (although numerous discrepancies appear in the literature) the possible elevation of mood attending a rise in CSF norepinephrine may be the most potent factor. None of the families reported loss of seizures with stimulation but all found the patients more alert, less irritable and generally easier to get on with. A direct relationship between the CSF levels of norepi and depression (fall of norepi with increasing depression and vice versa) is documented in the literature.

Project Description:

Objectives:

1. To study causal mechanisms of epileptic seizures in man and other primates.

2. To study the electrographic characteristics of epiletogenic activity in the brain of man and other primates.

3. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

4. To make use of opportunities in diagnosis and therapy for the study of neurophysiological and neuropsychological problems.

Methods Employed:

1. Clinical neurological, contrast and radiologic examination.

2. Neurophysiologic examination (macro-and micro-electrode, NADH fluorometric methods).

3. Evaluation of changes in histology and CSF neurotransmitters.

4. Neuropsychological examination of speech and cognitive function.

Major Findings:

The results of the effect of chronic cerebellar stimulation upon intractable cerebral seizures is provided in the Summary.

Other studies did not progress due to the absence of the Principal Investigator during part of the year and the lack of support for the clinical service.

Proposed Course of the Project:

The following projects have been approved by the CRC NINCDS and will be pursued as the facilities and support permit:

a) Cerebellar stimulation in the treatment of medically intractable epilepsy - This project continues 73-N-02 J00.156 and has been initiated to control the placebo factors in cerebellar stimulation, confirm the CSF neurotransmitter findings of the preliminary study (with Laboratory of Clinical Science, NIMH) and effects upon sleep patterns (Adult Psychiatry Branch, NIMH).

b) Changes in CSF levels of neurotransmitters associated with stimulation of the cortical and subcortical structures in the human brain - It is proposed to study levels both of norepinephrine and dopamine before and after depth stimulation in the brain both from ventricular fluid (during stereotaxic procedures) and lumbar fluid (on the ward). (With Laboratory of Clinical Science, NIMH).

c) Tegmental (H-Field) lesions in the treatment of intractable incapacitating epilepsy - The clinical indications for and results of the procedure will be studied. In addition, the implanted electrodes will be used to study the effect of depth stimulation upon CSF neurotransmitter levels and speech and cognitive function.

d) Microelectrode studies of cortical epileptogenic foci in man - This study makes use of a unique device produced on the reservation to record and hold cortical units despite the gross movements of the exposed human brain. The relationship between unit activity and cortical spikes recorded with macro electrodes has never been adequately studied in human epileptic foci.

e) Study of localization and selective inhibition of speech and cognitive function in the human brain by electrical stimulation - Material is provided by cortical stimulation during epileptic craniotomies and depth stimulation from electrodes implanted for epileptic diagnosis or therapy of dyskinesias or thalamic pain.

f) Evaluation of NADH fluorometry as an adjunct to ECoG in the surgical localization of focal cerebral seizures - This makes use of a unique instrument designed on the reservation which permits scanning the entire operative field for changes in the characteristic fluorescence of NADH in response to spontaneous epileptic activity or electrical stimulation of the cortex. The present apparatus is not clinically useful since the results require prolonged manual analysis. It has not been possible to obtain financial support to automate the apparatus through contact mechanisms.

Publications:

Van Buren, J.M. and Fedio, P.: Functional representation on the medial aspect of the frontal lobes in man. J. Neurosurg. 44: 275-289, 1976

Rajjoub, R.K., Wood, J.H., and Van Buren, J.M.: Significance of Purkinje cell density in seizure suppression by chronic cerebellar stimulation. Neurol. 26: 645-650, 1976.

Wood, J.H., Ziegler, M.G.; Lake, C.R., Shoulson, I., Brooks, B.R. and Van Buren, J.M.: Cerebrospinal Fluid Norepinephrine Reductions in Man after Degeneration and Electrical Stimulation of the Caudate Nucleus. Ann. Neurol. 1: 94-99, 1977

Wood, J. H., Lake, C. R., Ziegler, M. G., and Van Buren, J. M.: Neurophysiological and neurochemical alterations during electrical stimulation of human caudate nucleus. J. Neurosurg. 46: 361-368, 1977.

Wood, J. H., Glaeser, B. S., Hare, T. A., Sode, J., Brooks, B. R., and Van Buren, J. M. (in press): Cerebrospinal fluid GABA reductions in seizure patients evoked by cerebellar surface stimulation. J. Neurosurg.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 00200-23 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Involuntary Movements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J.M. Van Buren, M.D.	Associate Chief	CN NINCDS
	P. Fedio, Ph.D.	Research Psychologist	CN NINCDS
OTHER :	C.R. Lake, M.D.	Clinical Associate	LCS NIMH
	C. Gillin, M.D.	Clinical Associate	AP NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH
Medical Neurology Branch, NINCDS

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda,Maryland 20014

TOTAL MANYEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No further work has been undertaken on this project due to the absence of the Principal Investigator.

Project Description:

The Project Description remains the same as before and work is planned to study the following with implanted electrodes:

1) Reexamine the effect of fronto-caudate stimulation on the lumbar CSF levels of norepinephrine and add studies of dopamine.

2) Study the effect upon ventricular levels of these transmitters of acute intrathalamic stimulation during thalamotomy.

3) Study the effect of low level intermittent fronto-caudate stimulation upon EEG sleep patterns.

4) Continue studies of the effects of depth stimulation upon speech and cognitive function as the opportunity arises.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 00304-22 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Lesions upon the Function and Structure of the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	J.M. Van Buren, M.D.	Associate Chief	CN NINCDS
OTHER:	R. C. Borke	Biologist	CN NINCDS
	C.-L. Li, M.D.	Neurophysiologist	SN NINCDS
	D. King, M.D.	Staff Fellow	CN NINCDS
	W. Schuette, E.E.	Electrical Engineer	DRS NIH

COOPERATING UNITS (if any)

EM Section, Institut für Hirnforschung, Zürich, Switzerland

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 3.1	PROFESSIONAL: 1.0	OTHER: 2.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(Done at Institut Für Hirnforschung, Zürich) 1) Using an acetylcholinesterase (AChE) staining method in the spinal cord of the weakly electric gymnotid Sternarchus albifrons two populations of spinal neurones were found. The peripheral neurones related to the spinal musculature showed a positive staining reaction. The neurones (electromotor neurones) related to the electric organ (consisting simply of axons of these cells) lay about the central canal of the spinal cord appeared to lack dendrites and were AChE negative. 2) Thin section and freeze-etch studies in the subfornical organ of neonatal kittens have shown gap junctions on neuritic filapodia as well as on processes with immature synapses. A new type of "belted" gap junction of large size was found between early ependymal elements. 3) A comprehensive atlas of the freeze-etch morphology of the vertebrate nervous system was prepared.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is directed toward the study of basic neuroanatomy and correlated neurophysiology making use, where possible, of human pathological material and the opportunities afforded by the operative treatment of neurological disease.

Methods Employed:

The new construction will remove our large processing laboratory and our ability to serially section whole human brains. Our hopes of being able to pursue topographical neuroanatomical studies using techniques to demonstrate specific enzymes (i.e., the glutamic acid decarboxylase lable of Roberts) has not proved feasible due to the lack of local personnel and the logistical difficulties of distant collaboration. Thus, we have turned to thin section and freeze-etch electron microscopy which seems within the capabilities of our personnel and facilities.

Major Findings:

No work has been completed in our present facilities. Work done in Zürich by one of us is cited in the summary.

Proposed Course of Project: The emphasis in the past year has been upon personnel training.Work has gone slowly and the lack of a freeze-etch apparatus has delayed this aspect. The two projects under study are as follows:

a) Correlation of the action potential and the morphology of regenerating nerve.

i. Normal studies: Correlations between fiber size spectra and the action potentials are being prepared for peripheral sensory and mixed nerves and autonomic nerves. Variations in immersion fixation are being evaluated as it is desirable to fix the nerve in situ in the lucite stimulation-recording chamber. Perfused material serves as the control.

ii. Late results of nerve suture: In July 1976, the saphenous and post. tibial nerves in 6 cats were sectioned and resutured with 10-0 suture under the microscope. This material will be studied both distally and proximally to the suture 9-12 months after resuture. Especial interest will be paid to fiber diameter spectra and the regenerating nodes of Ranvier. (A particle size analyser has been obtained from Dr. Coulombre). It is hoped to use freeze-etch technique in this work.

b) Studies of the experimental meningocerebral cicatrix.

i. Normal studies: The junction of the cortex and the leptomeninges had been studied in perfused material in thin section and also some 20 satisfactory freeze-etch replicas of the pia-arachnoid were prepared with the kind loan of equipment by Dr. Fex (Laboratory of Neuro-otolaryngology).

ii. Studies of the meningocerebral cicatrix secondary to operative exposure: In September-October 1976 the cerebral cortex of 6 cats were exposed for 1 hour with intermittent irrigation. In addition, one area in each animal was exposed to 355 nm UV irradiation at energy levels between 100 and 700 mW/cm ^{2}. This material will be examined in 9 - 12 months and it is hoped to use both thin and freeze-etch methods.

When the freeze-etch facilities are available it is proposed to search for changes in junctional morphology associated with neuronal hyperactivity in experimental epileptic foci and in human epileptic cortex.

Publications:

Sandri, C., Akert, K., Kristol C., Van Buren, J.M. and Bennett,M.V.L.: AChE-positive and -negative motoneurones in the spinal cord of Sternarchus albifrons. Brain Res. 111: 157-162, 1976.

Sandri, C., Van Buren, J.M., and Akert, K. (1977). Membrane morphology of the vertebrate nervous system. A study in freeze-etch technique. Prog. Brain Res. 46: 1-384. Elsevier, Amsterdam.

Van Buren, J.M., Akert, K., and Sandri, C. (in press): Neuritic growth cone and ependymal gap junctions in the feline subfornical organ during early development. J. Cell Res.

Van Buren, J.M., Borke, R.C. and Modesti, L. M.: Sensory and nonsensory portions of the nucleus "ventralis posterior" thalami of chimpanzee and man. J. Neurosurg. 45: 37-48, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01245-12-CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Fedio	Psychologist	CN NINCDS
	W. Sheriff	Computer Programmer	TD NINCDS
OTHER:	M. Buchsbaum	Research Medical Officer	AP NIMH
	J. Van Buren	Associate Chief	CN NINCDS
	A. K. Ommaya	Acting Chief	SN NINCDS

COOPERATING UNITS (if any)

Section on Technical Development, NIMH; Adult Psychiatry Branch, NIMH

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Central processing of information by the human brain was monitored by averaged evoked response techniques. The electrographic recording of left and right brain activity during learning and perception in normal subjects was compared with that of neurosurgical patients. Suspect disturbances in brain-behavior relations in psychiatric patients were evaluated, relating left brain dysfunction to ideational disorders, right brain to emotional problems.

Serial No. Z01 NS 01245-12-C

Project Description:

Objectives: To identify brain mechanisms in man which regulate perception, and the storage and retrieval of information; to evaluate the significance of brain dysfunction in psychiatric patients by electrophysiological techniques.

Methods Employed: A series of language and spatial tasks, designed to evaluate left or right brain processes, were used. Electroencephalographic (EEG) activity was recorded from scalp electrodes positioned over the posterior temporal-parietal regions of the left and right hemispheres. Included for study were neurosurgical patients who had undergone unilateral removals of the temporal lobe, and psychiatric patients exhibiting affective or ideational thought disorders.

Major Findings: All electrographic test runs were conducted off-line and the evoked potential data for cognitive parameters is currently being processed. The study involving neuropsychiatric patients is in progress.

Significance to Biomedical Research and the Program of the Institute: Behavioral data available from epileptic patients following unilateral temporal lobectomy reveal significant perceptual and learning deficits which are related to the laterality of surgery and to the specific character of the material. The technique employed in this project affords a more precise method for outlining cortical and subcortical systems in the human brain which mediate learning and memory. The research also provides physiologic and behavioral data for the comparison of neurologic and psychiatric patients in order to identify possible brain dysfunctioning in schizophrenia or psychosis.

Proposed Course of the Project: To develop additional neuropsychological tasks and biometric programs for EEG analysis with wider application to patients with different neurologic or psychiatric disorders.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01424-11-CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Fedio	Psychologist	CN NINCDS
OTHER:	J. Van Buren	Associate Chief	CN NINCDS
	A. Ommaya	Acting Chief	SN NINCDS

COOPERATING UNITS (if any)
Department of Psychiatry, Massachusetts General Hospital, Boston, Massachusetts; Department of Neurology, Georgetown University Hospital, Washington, D. C.

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Emotional and sensory characteristics are studied in patients with temporal lobe epileptic foci. Patients and raters independently complete true-false questionnaires which probe specific behavioral and emotional traits, and permit analysis of distortions in self perception. In a separate investigation of sensory disturbances, the threshold for detection and recognition of olfactory and gustatory stimuli was established. Temporal epileptic patients are compared with matched normal subjects and patients with other neurologic illnesses. Patients with a right temporal focus are compared with left temporal epileptics. Statistical analyses are employed to codify behavioral and sensory profiles of right and left temporal epileptic subjects. The research examines the role of the anterior temporal lobe in establishing limbic associations and differences between the left and right hemispheres in regulating emotions and sensory experiences in man.

Project Description:

Objectives:

1. To identify personality profiles of epileptics with confirmed tempora lobe seizures. Although the relationship between limbic lesions and emotion disorders has been clinically suggested, most psychometric tests have been found to be 'insensitive' to the unique personality or behavioral disturbances displayed by individuals with temporal lobe disorders.

2. To evaluate sensory disturbances in olfactory and gustatory modalities as related to limbic or temporal lobe epilepsy. Whereas neuroscientific effort has traditionally investigated the role of the temporal lobe in mediating perception and integration of visual and auditory experiences, the Unit recently completed a study of olfactory and gustatory deficits produced by temporal lobe lesions. In collaboration with Drs. Henkin and Comiter (Georgetown University Hospital) detection thresholds and perception for vapors and tastants were determined for epileptic patients who had undergone a unilateral left or right temporal resection.

Methods Employed:

1. Based on several reliable 'clinical signs', a 100-item personality questionnaire was designed: one form was completed by the patient while an alternate form of the same scale was filled out by a relative or reliable informant.

The questionnaire was administered to four groups, matched for age, educational and socioeconomic level. The temporal groups consisted of epileptic patients with unilateral EEG foci located in the left or right temporal lobe; none of the patients were being considered for surgery and all were being maintained on a standard regimen of anticonvulsant medication. Two control groups were selected, one group was composed of patients with peripheral neuropathologic disorders; another group contained normal individuals.

2. Detection thresholds (absence or presence of a stimulus) were established for 4 taste qualities (sweet, sour, bitter and salt) and 4 vapors (pyridine, nitrobenzene, thiophene and amyl acetate). The subjects were also instructed to identify each tastant and vapor.

Major Findings:

1. The personality profile of the epileptic group was abnormal in comparison with the self-rater inventory of both control groups. Moreover, the left and right temporal patients were also judged by their raters to demonstrate a higher degree of unusual or abnormal behavior. Although seizure frequency was not related to degree of emotional disturbance, duration (years) of seizure disorder affected abnormal personality profiles, that is, the

'longer' the subject had epilepsy, the more likely he demonstrated aberrant behavioral traits.

These data complement the classic asymmetry of cognitive functions assigned to the left and right brain, and extend these observations to an asymmetry of emotional processing by the left and right cerebral hemisphere.

2. The left and right temporal epileptic patients were unimpaired in detecting the presence or absence of each tastant and vapor by psychophysiological methods. In contrast, both temporal groups had appreciable trouble in identifying each taste and smell stimulus. These data extend the perceptual role of the temporal lobe beyond visual and auditory experiences, to include the more basic olfactory and gustatory sensations.

Significance to Biomedical Research and the Program of the Institute: By identifying specific behavioral sequelae of a temporal lobe focus, these observations further neuroanatomical understanding of emotional processes. The results may be interpreted as a consequence of enhanced sensory-limbic associations. This interpretation regarding the effects of temporal lobe epilepsy in human subjects is consistent with extensive animal experimentation on sensory-limbic disconnections. The findings quantitatively support an asymmetry of emotional processing within the right and left hemisphere of man.

Proposed Course of the Project: Testing of additional psychiatric and neurologic contrast groups (nontemporal epileptics) is planned.

Publications:

Bear, D. and Fedio, P.: Quantitative analysis of interictal behavior in temporal lobe epilepsy. Arch. Neurol. 34: 454-467, 1977.

Henkin, R. I., Comiter, H. and Fedio, P.: Taste and smell dysfunction following temporal lobectomy. Proceedings of the Annual Meeting of the American Neurological Association, Chicago, Illinois, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01658-10 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Hemispheric Development and Specialization of Intellectual Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Fedio	Psychologist	CN NINCDS
	J. Van Buren	Associate Chief	CN NINCDS
OTHER:	G. R. Frederick	Psychologist	CN NINCDS

COOPERATING UNITS (if any)
Neurological Surgery, University of Washington, Seattle, Washington; National Technical School for the Deaf, Rochester, New York; Laboratory of Psychology, NIMH

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	0.6	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The disabling effects of brain damage in man were evaluated on a broad range of perceptual, learning and memory functions. Changes in the intellectual behavior of neurologically handicapped individuals were evaluated before and after brain surgery and during electrical stimulation of the surface and depths of the brain. In contrast to these cases with confirmed brain injury, the effects of peripheral, sensory deficits were assessed in terms of possible neuropsychological dysfunctioning, and communicative disorders.

Project Description:

Objectives:

1. Outline brain mechanisms which support speech and language functions, and code information to be held for immediate (short-term) or for delayed (long-term) memory; assess the effects of injury to these brain regions on communication skills and memory.

2. Compare the effects of brain injury with abnormal developmental conditions wherein there are sensory restrictions to language, as with congenital deafness.

3. Examine the role of the temporal lobe in guiding visual behavior under altered or distorted conditions.

Methods Employed:

1. The laterality and general outline of cortical zones instrumental in basic speech and language functions were mapped by stimulation of the cortex during neurosurgical treatment of epileptic patients. The behavioral tests utilized photographs of common objects with instruction to name the object.

2. In collaboration with Dr. George Ojemann at the University of Washington, a study was designed to evaluate the role of cortical speech areas in grammatical or syntactical organization of language. Electrical stimulation was applied over the frontal (Broca) speech cortex during performance by patients on various tests of grammar.

3. A standard prismatic adaptation test design and apparatus was used, creating a perceived displacement of visual targets. Subjects are trained under a variety of adaptation procedures in tracking visual objects and in remembering their spatial position.

4. The cerebral organization and lateral representation of language in the brain of deaf individuals will be evaluated by non-invasive, behavioral techniques. The testing procedure uses a tachistoscope, an apparatus which projects visual material at high speed (msec) onto the left or right visual fields, which in turn transmit the information directly to the left or right brain for processing. Accuracy of recognition for stimuli appearing in the left or right field reflects the efficiency of processing by left or right hemisphere. A variety of stimuli will be used to assess cerebral processing (printed words, designs, manual alphabet and sign language).

Major Findings:

1. The ability to identify objects and to remember their names was studied during electrical stimulation of the exposed surface of the brain in patients undergoing surgery for the relief of epilepsy. This speech mapping procedure identifies by stimulation those cortical areas of the

brain that are essential for the preservation of language. While there has been concerted effort to map the 'classic', posterior cortex, more recently, our team has had the opportunity to explore the more anterior cortical regions, in an effort to identify the production mechanisms of speech.

The general findings conform to established observations that language and related verbal processes rely upon an intact left brain. In the posterior temporo-parietal regions (Wernicke's area) of the left hemisphere, we were able to map a distinct zone which is indispensable for identifying verbal material. There was a disruption of basic language processes, the patient being unable to name simple objects. Stimulation of the left frontal cortex (Broca's area) also interfered with object naming, albeit to a lesser degree. Stimulation of Broca's area did not prevent the patient from recognizing or remembering objects but from stating the name until after stimulation was terminated, taking the appearance of blocked phonation or articulation. The patients experienced a similar blockade or interference with speech production during stimulation of the supplementary motor area.

Whereas the classic deficit in language with Wernicke's aphasia emerges as an inability to name, this process is preserved when Broca's area is stimulated. Instead, speech production is rendered inoperative. A significant disruption in the ability to speak was also evident from stimulation of the supplementary motor cortex. Although there were sharp differences between anomia and posterior stimulation, and dysarthria or arrest from more anterior stimulation, stimulation over the medial frontal and supplementary motor cortex produced similar disturbances in speech. Surprisingly, these patterns also resembled the type of speech impairment produced by stimulation of the frontostriatal region of the human brain.

2. The effects of unilateral right and left temporal lobectomy on adaptation to a right displacement of the visual field in humans was studied. The right temporal subjects showed less adaptation than control subjects in pointing to a visible target at the far right limit and at midpoint of the right visual hemifield. On testing for short-term retention of graded linear pointing before and after prismatic adaptation, the control subjects exhibited a shift to the right of their pointing estimates before adaptation and a further shift to the right following adaptation to prismatic vision. The right temporal lobectomized patients differed and showed on memory testing a tendency to shift to the left. The left temporal patients exhibited considerable accuracy for retention of pointing on baseline performance; following adaptation to prisms, these epileptic subjects pointed left of their estimates. These findings indicate that subjects with right temporal excisions were more impaired in visuo-adaptive behavior than subjects with left temporal excisions in perceptuo-motor performance under conditions of prismatic adaptation. Both brain-lesioned groups differed from unoperated subjects in retention of graded linear pointing on overall memory for the spatial location of visual targets.

3. The study of the operational efficiency of the 'language brain' in

individuals who have been deprived of auditory experiences by congenital deafness, is in the pilot phase. Individuals who became deaf before language skills were acquired (pre-lingual) present unusual linguistic characteristics that are frequently seen in neurologically impaired individuals. Whether the loss is due to the absence of auditory input into the language brain or to the absence of cross modal integration, that is, auditory-visual, requires further neuropsychological investigation.

A preliminary review of certain developmental and behavioral features of a select population of deaf individuals revealed atypical distribution with regard to handedness. Whereas the estimate for left-handed individuals in the general population runs between 5-12%, the initial survey of a deaf population (college students) yielded 16% left-handed subjects. This is interesting along several lines, that is, whether the high rate of deaf sinistrals is due to some underlying brain injury or whether the reliance by deaf individuals to rely heavily on visuo-spatial communication (lip reading, finger spelling, signing) encourages development of the right brain.

Significance to Biomedical Research and the Program of the Institute: These investigations contribute to the basic understanding of the development and organization of structural-functional relationships in the human central nervous system. This research advances clinical knowledge of the relationships between brain dysfunctions and amnesia, dysphasia, dyslexia and kindred communicative disorders.

Proposed Course of the Project: A battery of tests is being designed to examine adaptive strategies used by neurologic or sensory handicapped patients to compensate for visuomotor or language disorders. Visual and auditory tasks will be developed to further delineate immediate and long-term memory impairment in patients with lateralized cortical and subcortical lesions. Parallel studies of interhemispheric relations will be made during deep brain stimulation and during cortical stimulation of patients in the neurosurgical operating suite.

Publications:

Van Buren, J. M. and Fedio, P.: Functional representation on the medial aspect of the frontal lobes in man. J. Neurosurg. 44: 275-289, 1976.

Frederick, G. C., Quinn, C. P., Gillin, J. C. and Wyatt, R.J.: Deprivation of rapid eye movement sleep and nesting behavior in rats. Physiol. & Behav. 18: 341-344, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02213-02 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neuron Response to Axon Injury in the Immature and the Mature Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Rosemary C. Borke	Biologist	CN NINCDS
OTHER:	M.W. Brightman, Ph.D.	Section Head	LNNS NINCDS

COOPERATING UNITS (if any)

Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.1	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The results indicate distinct differences with maturation upon the neuronal-glial reaction of the hypoglossal nucleus to axonal injury in rats. Up to some 10 days postnatal, the neuronal membrane engulfs the presynaptic terminals in contact with its membrane and the proliferative glial phase is delayed to 1-3 weeks postoperative. In the mature neurone (21+ days postnatal) the microglia proliferate earlier and lift the presynaptic terminals off the neuronal somata.

Project Description:

Objectives:

1. To compare the ultrastructural features of retrograde responses to nerve crush and ligation in a system capable of regeneration (three-week-old rat) with a system incapable of regeneration (one-week-old rat).

2. To study the sequence of events of the progressive changes in the perikaryon capacity to respond to axonal injury.

3. To study the ultrastructural mechanism by which neurons are switched to a different metabolic program from that operating during the cortical maturation period of neurons.

4. To compare the glial reaction to axon injury in immature and mature animals.

5. To correlate the cell body responses with axonal regeneration in cells capable of regeneration (young adults) and cells incapable of regeneration (newborn - 7 days).

6. To study the membrane events of the axon and cell body of neurons subjected to nerve crush and ligation.

Methods Employed:

1. Surgical techniques of ligation and nerve crush of hypoglossal nerve in rats.

2. Transmission Electron Microscopy of the ultrastructural changes associated with axon regeneration and retrograde resection of the cell body.

3. Electron staining using tannic acid to delineate alterations in surface membrane coats of synapses on soma and dendrites of hypoglossal nucleus cells.

4. Freeze fracture techniques of hypoglossal nucleus and nerves in normal and injured neurons to study the membrane events associated with the retrograde responses.

5. Neurophysiological stimulation techniques as needed to test functional regeneration of axons.

Results:

Albino male rats 7, 10 and 21 days old were subjected to hypoglossal nerve crush and another group to nerve ligation. The hypoglossal nuclei on the operated and nonoperated sides were compared after survival time of 1-3 days, 7-13 days and 20-40 days (total of 155 experimental animals to date), 84 control and sham operated animals of each postnatal age and corresponding postoperative survival have served as comparison. All animals were perfused and prepared for thin section EM.

Results indicate a differing reaction with age.

In the mature neuron (21 day animals) the microglia proliferate 1-3 days postoperatively and invade the hypoglossal nucleus to surround the reacting cell soma. Presynaptic nerve terminals are lifted off the neuronal membrane. (1-7 days).

In immature neurons (7 and 10 day animals) the proliferative glial phase is delayed (7-20 days) and during the early postoperative period (1-7 days) the neurons appear to send out cytoplasmic protrusions which surround dendrites and presynaptic terminals in contact with its membrane. The fate and time course of these protrusions is currently under study.

Proposed Course of Project:

Freeze-etch studies of these reactive changes are planned.

Publications: None

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02214-02 CN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of 3, 5, 3' Triiodo-L-Thyronine on Cell Body and Axons Following Injury to Hypoglossal Nerve

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Rosemary C. Borke	Biologist	CN NINCDS
OTHER :	M. W. Brightman, Ph.D.	Section Head	LNNS NINCDS

COOPERATING UNITS (if any)

Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS

LAB/BRANCH

Clinical Neurosciences

SECTION

Functional Neurosurgery

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

At small doses of triiodo-L-thyronine the effects were indeterminate. At large doses the animals died. The project was terminated.

PHS-6040
(Rev. 10-76)

Project Description:

(See last years description of objectives and methods)

Major Findings

The hypoglossal nerve was crushed in 15 rats 10 days postnatal and 15 rats 21 postnatal. In each group 5 rats were perfused on the 3rd, 7th and 20th day post surgery. All rats received triiodothyronine (T3) daily. The dosage varied from 0.5, 1.0, 7.0 and 10.0 mg./100gm body weight. The two larger doses were incompatible with survival.

No changes in the neuronal or glial reaction to axonal crush was evident on thin section examination in the animals receiving the lower dosages.

Proposed Course of the Project: Project was terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02207-02 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
NADH fluorescence and $[K^+]_o$ in the gerbil cortex following carotid artery occlusion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	B. Vern, M.D.	Clinical Associate	CN NINCDS
OTHER :	N. Mutsuga, M.D.	Visiting Fellow	CN NINCDS
	W.C. Whitehouse	Section Head	PSD CC

COOPERATING UNITS (if any)
Television Engineering Section, Clinical Center

LAB/BRANCH
Clinical Neurosciences

SECTION
Clinical Neurophysiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was terminated.

PHS-6040
(Rev. 10-76)

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02208-02 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Cortical Fluorescence Changes, Epileptic Activity and Blood Flow

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	D.W. King, M.D.	Clinical Associate	CN NINCDS
OTHER :	W.H. Schuette	Biomedical Engineer	BEI DRS
	W.C. Whitehouse	Section Head	PSD CC

COOPERATING UNITS (if any)
Biomedical Engineering Branch, Division of Research Services
Television Engineering Section, Clinical Center

LAB/BRANCH
Clinical Neurosciences

SECTION
Clinical Neurophysiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An attempt was made to determine the validity of our present method for measuring NADH fluorescence over relatively large areas of the cat cortex. It was found that with our present system of calibration, a transient increase in blood pressure (BP) resulted in changes which we would normally interpret as an increase in metabolic activity. When calibration on the basis of a transient rise in BP was used, no changes in "metabolic activity" were present during electrical stimulation or seizure. Since it is more likely that transient changes in BP cause an increase in blood flow than an increase in metabolic activity, we suspect that our present method for determining metabolic activity (changes in NADH fluorescence) is severely contaminated by changes in blood flow.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To attempt to determine the validity of our present method for measuring NADH fluorescence over relatively large areas (>1 mm^2) of the cat cortex.

Methods Employed: Adult cats were anesthetized with Na pentobarbital or Ketamine plus ether, immobilized with Flaxedil, and artifically ventilated with moisturized O_2. Bipolar silver ball electrodes with a 1 mm tip separation were used for cortical stimulation and topical penicillin was applied by a saturated pledget to produce seizure activity. DC electrocorticography was recorded using Ag-AgCl cotton wick electrodes. The cortex was illuminated with ultraviolet light (366 nm) and fluorescence was measured using a television fluorometer previously described. (Schuette et al, Med Instr. 8: 331-333, 1974). In half of the animals, reflected 366 nm light was used as a reference. In the others, 529 nm light from the fluorescence of intravenously injected fluorescein was used as the reference (Vern et al, Brain Res. 98: 405-409, 1975). Calibration between the cortical fluorescence channel (430-470 nm) and the reference channel was carried out using the following methods: 1) a 20% neutral density filter in front of the exciting HV light; 2) a bolus intravenous injection of saline and 3) a transient increase in blood pressure produced by the inflation of an intra-aortic Fogarty Catheter.

Major Findings: 1) When a 20% neutral density filter gave equal changes to the cortical fluorescence and reference signals, the difference between the cortical fluorescence and the reference (either 366 or 529) decreased during electrical stimulation, seizures, or transient increases in blood pressure. 2) When the injection of normal saline gave equal changes to the cortical fluorescence and reference signals, cortical stimulation and seizures resulted in a decrease in the difference between the cortical fluorescence and the reference, but by a smaller amount and less consistently than when the 20% neutral density filter was used. 3) When a transient increase in blood pressure resulted in equal changes in the cortical fluorescence and reference signals, there was essentially no change in the difference between the cortical fluorescence intensity and the reference intensity during cortical stimulation or seizures. 4) The changes in cortical fluorescence which occurred when the 20% neutral density filter or the normal saline were used for calibration could not be correlated topographically with DC potential shifts when measurements were taken at different points in relation to the center of the epileptic focus.

Conclusions: Our results suggest that either a transient increase in BP causes an increase in metabolic activity or the measurement of NADH fluorescence over the cat cortex is severely hindered by changes in blood volume in the field. Because we think it is more likely that transient changes in BP cause an increase in blood flow than an increase in metabolic activity and because the cortical fluorescence did not correlate with the DC potential shifts, we suspect that the present method is severely contaminated by changes in hemoglobin concentration in the field secondary to changes in blood flow during cortical stimulation and epileptic activity.

Proposed Course of the Project: Project is provisionally completed and a publication is in preparation.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02210-02 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Amino Acid Responses of Somatosensory Cortical Neurons in Evolving Acute Epileptic Foci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J.B. Macon, M.D.	Clinical Associate	CN NINCDS
	D.W. King, M.D.	Clinical Associate	CN NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Neurosciences

SECTION
Clinical Neurophysiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.8	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Feline primary somatosensory cortical neurons were studied with compound multibarrell micropipettes using the technique of combined penicillin and amino acid iontophoresis. Units had inhibitory responses to GABA, ALA and GLY and excitatory responses to HC and GLU. Topical penicillin, strychnine or bicuculline failed to consistently antagonize amino acid responses of units at cortical depths of 1-2mm. Penicillin iontophoresis (100-500nA) resulted in reversible nonspecific antagonism of all three inhibitory agents in order of potency GLY>ALA>GABA. Penicillin iontophoresis in higher dosage resulted in development of a micro epileptic focus in which decreased amino acid responses were observed. Penicillin iontophoresis in lower dosage resulted in enhanced physiological responses which were not correlated with decreased amino acid responses. Amino acid antagonism by penicillin appeared unlikely to be the primary mechanism by which penicillin produced epileptic foci.

Project Description:

Objectives: To determine if amino acid antagonism or facilitation was the primary mechanism underlying penicillin-induced acute epileptic foci.

Methods Employed: Extracellular single unit activity in feline somatosensory cortex was studied with compound seven-barrelled micropipettes. Convulsants and putative amino acid neurotransmitters were delivered by iontophoresis simultaneously with unit recording. Penicillin or other convulsants were applied topically in concentrated solutions in some experiments.

Major Findings: Inhibitory responses were observed to GABA, alanine and glycine and excitatory responses to homocysteic acid and Glutamate. Quantitative estimates of amino acid sensitivity were determined from the ED50 of dose-response curves. Topical penicillin, strychnine and bicuculline failed to consistently decrease amino acid inhibitory responses in topical foci at 1-2 mm cortical depths. Bicuculline iontophoresis (100 nA) antagonized GABA and ALA, but not GLY responses in most neurons. Strychnine iontophoresis (100 nA) antagonized GLY and ALA, but not GABA responses. Penicillin iontophoresis (100-500 nA) reversibly and nonspecifically antagonized all three inhibitory amino acids in the inverse order of potency GLY>ALA>GABA. Penicillin iontophoresis in high dosage (1-2 μA) resulted in evolution of microepileptic foci with decreased amino acid responses detected prior to the onset of unit bursts or surface spikes. Penicillin iontophoresis in low dosage (≤ 100 nA) resulted in enhanced physiological responses which were not correlated with decreased amino acid responses. Penicillin iontophoresis did not enhance responses to the excitatory amino acids HC or GLU. No modification of endogenous inhibition by penicillin iontophoresis could be demonstrated, although a decreased in post VPL stimulation inhibition in topical penicillin foci was observed.

The data indicated that amino acid response alteration by penicillin was unlikely to be the primary mechanism of penicillin epileptogenesis. Decreased amino acid sensitivity might be useful marker to identify neurons directly affected by penicillin in the evolving epileptic focus.

Proposed Course of the Project: This project has been completed and two manuscripts are ready to be submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02211-02 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Amino acid responses of hyperactive neurons in the deafferented spinal trigeminal nucleus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: J.B. Macon, M.D. Clinical Associate CN NINCDS

COOPERATING UNITS (if any)
Veterinary Resources Branch, Division of Research Services

LAB/BRANCH
Clinical Neurosciences

SECTION
Clinical Neurophysiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.4	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The amino acid responses of neurons in the cat spinal trigeminal nucleus were studied by iontophoresis using multi-barrelled micropipettes in twenty cats, two to four weeks following unilateral retrogasserian (rhizotomy). A population of deafferented spontaneously hyperactive neurons (Type 2) was identified with grossly decreased responses to exogenous amino acids. The amino acid hyposensitivity was nonspecific for both inhibitory (GABA, Glycine or Alanine) or excitatory (Homocysteic acid, Glutamate or Aspartic acid) agents, except in a small subgroup (Type 2b) which was specifically hyposensitive to GABA. Silent (Type 1) or bursting (Type 3) neurons were found to have normal amino acid response compared to the intact spinal trigeminal nucleus. The results provided no support for Cannon's law of denervation supersensitivity in the central nervous system. Amino acid hyposensitivity was proposed as a physiological property of central neurons undergoing transneural atrophy.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To distinguish between denervation supersensitivity and transneural atrophy as mechanisms of deafferentation hyperactivity in the feline trigeminal nucleus following retrogasserian trigeminal rhizotomy.

Methods Employed: Six-barrelled compound micropipettes were used to record extracellular single unit activity in the cat spinal trigeminal nucleus with simultaneous iontophoresis of putative amino acid neurotransmitter agents. Unilateral retrogasserian trigeminal rhizotomies were performed by the subtemporal approach in two cats two to four weeks prior to recording. The contralateral intact nucleus and six normal nuclei were studied to determine control responses.

Major Findings: Intact trigeminal nuclei neurons were found to be inhibited by GABA, Glycine and alanine in low doses (<40 nA). Deafferented neurons were divided into three groups based on spontaneous firing patterns. Type 1 (silent) and Type 3 (bursting) neurons exhibited normal amino acid responses to iontophoresis. Type 2 (continuous firing 20-50 hz) neurons had grossly decreased responses to all amino acids tested both inhibitory and excitatory. A small subgroup (Type 2b) had selectively decreased responses to GABA ($p<.001$). All hyperactive neurons encountered had dramatically decreased sensitivity to GABA. The results were inconsistent with Cannon's law of denervation supersensitivity. Transneural atrophy was suggested as the most likely explanation of these observations to account for both deafferentation hyperactivity and amino acid hyposensitivity.

Proposed Course of the Project: This project has been completed. At the time of preparation of this report efforts to confirm these findings in Rhesus monkeys following retrogasserian rhizotomy were in progress. A manuscript has been completed and is about to be submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02212-02 CN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Features and Ictal Patterns in Epileptic Patients with EEG Temporal Lobe Foci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	D.W. King, M.D.	Clinical Associate	CN NINCDS
	C. Ajmone Marsan, M.D.	Branch Chief	CN NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neurosciences

SECTION

Clinical Neurophysiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The clinical features and subjective ictal phenomena of 270 epileptic patients with electrographic temporal lobe foci are reported. The ictal manifestations observed in 199 of these patients are described and compared with those observed in epileptic patients with extra-temporal foci reported previously. Sixty-four per cent of the patients reported historical data of possible etiological significance, but one could be reasonably certain of the etiology in only 27%. Eighty-three per cent of the patients reported auras, 80% exhibited localized motor activity, and 95% demonstrated automatic behavior. When compared to patients with extra-temporal foci, the patients reported here had a higher incidence of visceral (41%), complex (52%), and olfactory-gustatory (13%) auras and of automatic behavior. On the other hand, no ictal phenomenon was unique to patients with temporal localization. The data do not support the concept of an association between "temporal lobe epilepsy" and violence.

Project Description:

Objectives: The ictal clinical manifestations of "temporal" epilepsy are extremely complex and variable in different patients or in the same subject at different times. This project is an attempt to analyze in detail such manifestations and to correlate them with the actual localization of the inter-ictal electrographic signs.

Methods Employed and Major Findings: This study utilizes over 200 patients. These are selected from the files of our laboratory on the basis of a) presence of EEG epileptiform activity localized within one or both temporal regions; b) availability of reliable and detailed repeated observations and description of seizure episodes. The study is still at the stage of data collection and tabulation: no major findings are available.

Proposed Course of the Project: This project is completed. Two papers have been accepted for publication and should appear in press before the end of this fiscal period.

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U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02268-01 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Topography of Cortical Fluorescence Changes in Focal Epileptiform Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	D.W. King, M.D.	Clinical Associate	CN NINCDS
OTHER :	J.B. Macon, M.D.	Clinical Associate	CN NINCDS
	W.H. Schuette	Biomedical Engineer	BEI DRS
	W.C. Whitehouse	Section Head	PSD CC

COOPERATING UNITS (if any)
Biomedical Engineering Branch, Division of Research Services
Television Engineering Section, Clinical Center

LAB/BRANCH
Clinical Neurosciences

SECTION
Clinical Neurophysiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.6	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A topographical analysis of the electrocorticographic DC potential shifts and cortical fluorescence changes was undertaken in both the center and surrounding areas of a penicillin-induced focal epileptic process. A consistent correlation between cortical fluorescence changes and proximity to the center of the focus was not found. Also, a consistent topographical correlation between DC shifts and cortical fluorescence changes was not found. Cortical fluorescence changes were usually of greater amplitude when measured over large vessels than when no major vessels were in the field.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the changes in cortical fluorescence both at the center of an epileptic focus and in the surrounding cortical tissue to determine if these changes correlate topographically with electrocorticographic DC potential changes.

Methods Employed: Adult cats were anesthetized with sodium pentobarbital or Ketamine plus ether, immobilized with Flaxedil, and artifically ventilated with moisturized O_2. A penicillin focus was created by either the topical application of a 1 mm^2 pledget soaked in a paste of Na penicillin or by the intracortical injection of 2-5 microliters of a solution of 100,000 units of Na penicillin dissolved in 1 cc of distilled water. DC electrocorticography was recorded at the center of the focus and over the surrounding cortex using Ag-AgCl cotton wick electrodes. The cortex was illuminated with ultraviolet light (366 nm) and fluorescence was measured both at the center of the focus and over the surrounding cortex using a television fluorometer previously described (Schuette, W.H. et al, Med. Instr. 8: 331-333, 1974). Fluorescein was used as the reference (Vern, B.A. et al, Brain Res. 98: 405-409, 1975).

Major Findings:

1. During seizure activity, negative DC shifts were observed at the center of the focus and over the surrounding cortex. As the electrode was moved further from the cortex of the focus, the onset and peak of the negative shift was progressively delayed.

2. Cortical fluorescence changes were variable and could not be consistently correlated with the distance from the center of the focus or with the negative DC shifts.

3. Cortical fluorescence changes were usually (but not consistently) of greater amplitude when measured over large vessels than when no major vessels were in the field.

Conclusions: Definite conclusions can not be drawn. However, the lack of topographic correlation with the DC change plus the larger amplitude of changes over major vessels raise the possibility that much of the fluorescence change originally thought to be due to the conversion of NADH to NAD^+ is actually due to a change in blood volume associated with epileptic activity resulting in a change in absorption of the signal by the hemoglobin in the blood.

Proposed Course of the Project: This project is provisionally completed and a publication is in course.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02269-01 CN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Photic Flash Visual Evoked Potentials in Clinical Neurology and Neuro-Ophthalmology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	N. Olmos-Lau, M.D.	Clinical Associate	CN NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neurosciences

SECTION

Clinical Neurophysiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Analysis of morphology, amplitude and latency of visual evoked potentials in various neurological disorders.

Project Description:

Objectives: The use of visual evoked potentials (VEPs) in the diagnostic evaluation of neurologic patients is now becoming a common procedure in most Clinical Neurophysiology Laboratories. At the Clinical Center of NIH with active clinical evaluation of patients with multiple sclerosis and other complex neuro-ophthalmologic problems there is a need for the performance of this study on a routine basis. As a result of this, VEPs have been performed for the evaluation of various neurologic processess but mainly for the diagnosis of asymptomatic optic neuropathy of multiple sclerosis.

Methods Employed and Major Findings: Photic VEPs have been performed in 16 patients for diagnosis. In each case the morphology, latency and amplitude of the response was evaluated. The data along with future studies will be subjected to statistical analysis to provide more information on the changes of the VEPs in neurologic illness. This study is still in the stage of data collection. No major findings are available.

Significance to Biomedical Research and the Program of the Institute: This study will provide information on the clinical-electrophysiologic correlation of patients with cases asymptomatic optic neuritis in multiple sclerosis, and will provide data on the value of this diagnostic study. Other clinical neuro-ophthalmologic problems will be assessed as requested.

Proposed Course of the Project: This project should be completed within the next fiscal period.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02270-01 CN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical and EEG Features in Cases of Midline (Parasagittal) Epileptiform Abnormalities and Pathologic Lesions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	C. Ajmone Marsan, M.D.	Branch Chief	CN NINCDS
	N. Olmos-Lau, M.D.	Clinical Associate	CN NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neurosciences

SECTION

Clinical Neurophysiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	--

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A correlative study of clinical features and seizure patterns of 65 patients with electrographic evidence of central-vertex (Cz) epileptiform discharges and of 30 patients with pathological processes in the same region.

Project Description:

Objectives: The purpose of this study is to analyze and describe the clinical manifestations and ictal patterns in cases with midline electrographic epileptiform abnormalities and compare them with cases of known pathologic parasagittal lesions.

Methods Employed and Major Findings: Case selection from the files of our laboratory has yielded 40 cases of definite and 25 cases of probable epileptiform discharges restricted to the central-vertex (Cz) region. Such cases will be further classified on the basis of the analysis of the morphology, characteristics of distribution and other salient features of abnormalities. Information on ECoG, implanted electrodes and surgery results on these cases will be obtained. A group of 19 proven and 11 probable cases of pathologic parasagittal lesions have been selected as well to be analyzed and compared to the first group. The clinical features and seizure patterns of the patients in these two groups will be analyzed and correlated with the EEG features. This study is still in the stage of data collection.

Significance to Biomedical Research and the Program of the Institute: This study will provide useful information on the clinical spectrum of parasagittal seizures and might provide practical data on the relevance of restricted epileptiform discharges to the midline in the diagnosis of lesions in this area; results of surgical treatment will be assessed.

Proposed Course of the Project: This project will be completed within the next fiscal period.

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Developmental and Metabolic Neurology Branch
National Institue of Neurological and Communicative Disorders and Stroke
Roscoe O. Brady, Chief

The Branch has continued its high level of productivity and originality in the control and therapy of heritable disorders of the nervous system. A high-yield large-scale procedure has been devised and is now operational for obtaining highly purified glucocerebrosidase for enzyme replacement trials in Gaucher's disease. Sphingomyelinase, the enzyme lacking in Niemann-Pick disease has also been isolated in a highly purified form from human placental tissue. Furthermore, an animal model of this latter disorder has been developed by our laboratory. Another major advance in the therapy of hereditary neurological diseases last year was the demonstration that the blood-brain barrier can be temporarily altered to permit the entrance of enzymes into brain. Previously all enzyme replacement attempts along this line have been thwarted by the inability of exogenous enzymes to gain access to damaged nerve cells. Thus, the newly developed procedure offers the first hope for specific therapeutic measures in enzyme deficiency diseases that cause neurological dysfunction.

New simplified reagents for the diagnosis of Niemann-Pick disease, Krabbe's disease and Gaucher's disease that were designed and synthesized in this Branch are now being produced commercially and are widely available. These analogues of the accumulating lipids are also used for the detection of heterozygous carriers of these disorders and for the prenatal diagnosis of these diseases.

Other work in the Branch deals with the biochemical and physiological functions of gangliosides with particular regard to their role as biotransducers of environmental signals that control cellular metabolism. Gangliosides have now been shown to be important components in cell receptors for thyrotropin, chorionic gonadotropin and luteinizing hormone and insight has been obtained into the mode of interaction of these hormones with their target organs.

Additional investigations conducted by the Branch were directed towards the isolation, characterization and examination of the immunological properties of myelin-specific glycoproteins and their potential role in the pathogenesis of demyelinating diseases such as multiple sclerosis. Because these and other projects under investigation in the Branch involve a variety of approaches to the control and therapy of human disorders, this report is divided into the following specific categories:

I. Enzyme Replacement Therapy for Inherited Diseases

A. *Gaucher's Disease*

During the past year we have developed a novel high-yield large-scale procedure for the isolation of glucocerebrosidase, the enzyme lacking in patients with Gaucher's disease, from human placental tissue. Previous studies carried out by the Branch indicated that the intravenous injection of glucocerebrosidase causes a decrease in the quantity of glucocerebroside that had accumulated in organs and the blood of patients with Gaucher's disease. It was also discovered in our original enzyme replacement trials that the quantity of enzyme required by many of these patients was much greater than the amount that could be produced in a research laboratory.

By appropriate modifications of our original enzyme purification procedure, we have devised a successful method for the purification of glucocerebrosidase on a large scale in a form that is suitable for administration to humans. This method is now in operation. The procedure will be published in August so that it will be available to all investigators concerned with enzyme replacement for patients with Gaucher's disease.

B. *Niemann-Pick Disease*

We have developed a method for the purification of sphingomyelinase, the enzyme lacking in Niemann-Pick disease, also from human placental tissue. At the present time, it is very difficult to obtain sufficiently large quantities of this enzyme for replacement therapy trials in humans with Niemann-Pick disease. However, during the past year, we discovered that the administration of the compound known as AY-9944 to rats causes a dramatic reduction in the amount of sphingomyelinase in the liver and other organs in the recipients. The reduction in enzymatic activity is accompanied by an increase in tissue sphingomyelin, and Niemann-Pick like inclusion bodies appear in retinal neurons, an important pathological correlate of the disease in humans. AY-9944 does not affect the catalytic activity of the enzyme. The reduction of sphingomyelinase activity in the animal model is specific. It is caused by a decrease in the production of this enzyme. Thus, the pharmacologically induced animal model has all of the requirements for an examination of the effect of exogenous sphingomyelinase on the biochemical and pathological consequences attending this enzyme deficiency. We propose to use this animal model to explore the effect of enzyme replacement therapy in Niemann-Pick disease.

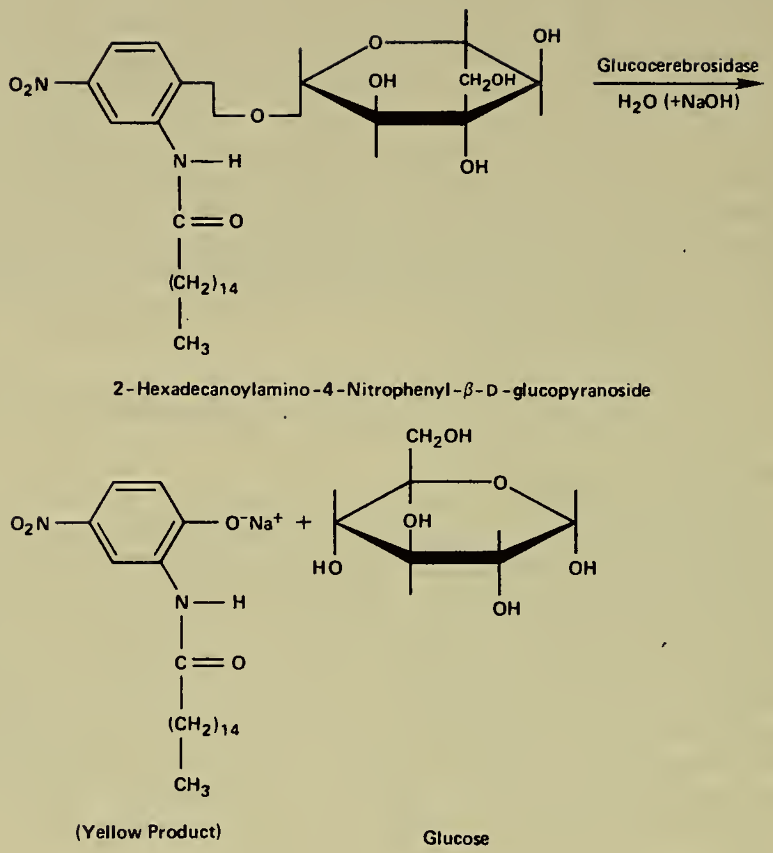
C. *Metabolic Disorders Affecting the Central Nervous System*

Since our original trial of enzyme replacement in patients with Tay-Sachs disease in 1972, it has been apparent that such individuals would not be benefited by the intravenous administration of the required enzyme without additional measures. This constraint is imposed by the blood-brain barrier which prevents the entrance of molecules as large as enzymes into the central nervous system. In order to examine the feasibility of temporarily opening the barrier, we carried out a survey of lysosomal hydrolyses in brain to determine if one of these might exhibit an appropriate level of activity so that superposition of exogenous enzyme would be readily and reliably detected over the basal enzyme activity. It was discovered that mannosidase was a likely candidate for such an investigation. Furthermore, individuals with insufficient mannosidase activity are known to be severely retarded mentally, so there is also a valid clinical rationale for such an undertaking. The requisite mannosidase was purified from human placental tissue. The blood-brain barrier was opened temporarily by intracarotid infusion of hypertonic solutions of mannitol or arabinose. Under these experimental conditions, a clear augmentation of mannosidase activity occurred in the brain after intravenous injection of the enzyme. This increase in brain mannosidase was fully equivalent to the physiological level of this enzyme. This first demonstration of the accessibility of exogenous enzyme to the central nervous system is a major accomplishment towards enzyme replacement therapy for the multiplicity of heritable disorders that cause brain damage.

II. Development of Universally Applicable Reagents for the Diagnosis of Hereditary Diseases.

The chromogenic analogue of sphingomyelin prepared by this Branch for the diagnosis of Niemann-Pick disease in 1975 is now commercially available at a fraction of the cost of the labeled authentic lipid previously required for this test. The chromogenic analogue of galactocerebroside prepared and tested here for the diagnosis of Krabbe's disease in 1976 will soon be offered commercially. The U. S. Government has applied for patents covering the synthesis and use of the Niemann-Pick reagent and similar applications will be filed for the galactocerebroside analogue. Patent rights have been assigned to the United States of America by the inventors.

Following the precedent of the successful development of chromogenic reagents for the diagnosis of Niemann-Pick disease and Krabbe's disease, we synthesized the chromogenic analogue of glucocerebroside, 2-hexadecanolyamino-4-nitrophenyl-β-D-glucopyranoside. This compound has now been shown to be a reliable substrate for the diagnosis of patients with Gaucher's disease and the detection of heterozygous carriers of this disorder. The reaction involved is illustrated in the following figure:



2-Hexadecanoylamino-4-Nitrophenyl-β-D-glucopyranoside

(Yellow Product)

Glucose

The availability of these three chromogenic analogues now places diagnostic capability for Gaucher's disease, Niemann-Pick disease and Krabbe's disease within the scope of all general clinical chemistry laboratories. Previously, the performance of these tests was restricted to research laboratory units, and expensive radioactivity labeled lipids were required for these tests.

III. Role of Gangliosides as Membrane Receptors for Environmental Signals

The demonstration that trophic hormones such as thyrotropin interact with gangliosides in thyroid cell membranes has been followed by a similar demonstration of chorionic gonadotropin with gangliosides in Leydig cell membranes. It is expected that a similar interaction will be found between luteinizing hormone and gangliosides in the appropriate target cell membranes. Chemical dissection of the reactive components of thyroid membranes indicates that there are probably two substances involved in the thyrotropin receptor, one a glycoprotein and the other ganglioside(s). Further insight into this reaction was obtained by examining the ganglioside composition of thyroid cell membranes. It has been found that the thyroid gland contains a minor novel ganglioside that has extremely high affinity for thyrotropin. This highly reactive ganglioside has been partially characterized. Studies are underway to elucidate its complete structure and to determine which portions of its molecule are involved in the binding of thyrotropin. These studies are expected to provide insight into the etiology into certain clinical conditions that include thyrotoxicosis, goiter, and some forms of hypothyroidism. It is not unreasonable to expect similar clinically relevant discoveries will follow the identification of specific gangliosides involved in the interaction of chorionic gonatropin and luteinizing hormone with their respective target organs.

IV. Myelination

During FY 77, a highly selective procedure was developed for the rapid isolation of myelin-specific glycoprotein from the central nervous system. We have thereby surmounted the critical impediment to the complete characterization of this novel component of myelin. In addition, we have shown that myelin glycoprotein is highly antigenic, and animals immunized with the whole myelin preparations develop anti-myelin glycoprotein antibodies. We are now investigating the role of this phenomenon in demyelinating conditions in experimental animals and in human disorders such as multiple sclerosis.

V. Epilepsy

Investigations with labeled phenobarbital indicated that this compound and its glucuronide conjugate are very slowly eliminated from the body. After a single intravenous dose, phenobarbital and its metabolites were still present in the urine after 16 days. There was no detectable elimination of phenobarbital through gastrointestional tract.

A comprehensive examination of the effect of anti-convulsant drugs on mental activity has shown that phenobarbital causes a marked impairment of short-term memory but the retrieval of information from long-term memory is unaffected. This observation illuminates the cause of the difficulty in learning that patients on anticonvulsive drug therapy experience.

The incorporation of the Section of Physiology and Metabolism into the Branch has provided expertise concerning the metabolic basis of seizures in epileptic animal models. Initial observations indicate that seizure-prone mice, chickens and hamsters may have deranged nucleotide metabolism certain areas of the brain.

VI. Mental Retardation

Following our demonstration of the extent of impaired intestinal absorption of copper in Kinky Hair Disease patients we have studied mechanisms by which copper can affect tissues in this disorder and in Wilson's disease. Radioisotope tracer studies using ^{67}Cu have provided insight into the pathogenesis of these disorders. It is concluded that Kinky Hair Disease is caused by a deficiency of copper and results in abiotrophy of the elastic tissue of blood vessels. In Wilson's disease, there is a deposition of excessive quantities of copper which is toxic resulting in severe dysfunction of the liver and brain.

VII. Mucopolysaccharidoses

Out of the six different types of mucopolysaccharidoses, only patients with Type V have normal mentality and histologically normal neuronal elements, although their white matter contains perivascular lesions similar to those in other types of mucopolysaccharidoses. Extended biochemical studies of the content and composition of glycosaminoglycans in the whole brain tissue and isolated mesenchymal tissue from the brain of the patients with mucopolysaccharidoses showed that the increase in brain glycosaminoglycans in types I-III depends largely on the contribution form the mesenchymal tissue, although the neural elements also showed some increase in glycosaminoglycan levels.

By administering oral corticosteroids to selected patients with mucopolysaccharidoses, we were able to modify the course of the disease with amelioration in the mental status and joint mobility. Concomittant biochemical studies on urinary and white cell glycosaminoglycans were conducted while the patients were on corticosteroids. Following the initial brief phase of increased urinary glycosaminoglycans, patients on maintenance corticosteroids showed a substantial reduction of glycosaminoglycans; there were also changes in the composition of glycosaminoglycans and in the distribution of their molecular weights. These studies have taken on additional importance because of recent attempts at enzyme replacement in Hunter's syndrome through subcutaneous implantation of cultured human skin fibroblasts. The recipients all had extensive immunosuppressive therapy including prolonged administration of steroids. Thus, any reported clinical effects in patients receiving such treatment must take into account the possible effect of the steroids *per se* on the course of the disease.

VIII. Stroke

Out of 171 patients with strokes, 22 had hyperlipoproteinemia as defined by the strict criteria established by WHO. Contrary to the previous belief that only type IV hyperlipoproteinemia carries increased risk for strokes, we found that the frequency of strokes in types IIa, IIb, III and IV corresponded to the frequency of these types in the population. In other words, patients with permanently elevated blood triglycerides, total cholesterol, or both of these components are at risk for strokes. Therefore, future therapeutic and preventive studies should include patients with all of the various types of hyperlipoproteinemias.

IX. Awards

The Branch was honored in FY 77 by the selection of the Chief of the Branch for the Sarah L. Poiley Award for the New York Academy of Sciences and he was elected Foreign Associate Member of the Argentinian Academy of Medical Sciences. Members of the Branch received the Special Recognition Award of the U. S. Public Health Service, and the Washington Academy of Sciences Award in Biological Sciences.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00706-18 DMN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Inborn errors of metabolism, cerebral degeneration and brain dysfunction of diverse etiology.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Anatole S. Dekaban, M.D.,Ph.D.	Associate Chief	DMN	NINCDS
Other:	George Constantopoulos, Ph.D.	Biochemist	DMN	NINCDS
	Michael P. Whyte, M.D.	Clinical Associate	DMN	NINCDS
	Daniel E. Britton, M.D.	Clinical Associate	DMN	NINCDS
	Jan K. Steusing	Research Assistant	DMN	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.7	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As evident from the title, this project incorporates a large number of diverse conditions, ranging from inborn errors of metabolism, to cerebral degenerations, to the brain dysfunction of diverse etiology. The unifying factors are cerebral involvement, determination of pathogenesis and therapeutic trials. The methodological approach includes clinical, and genetical apprasial, brain pathology through cerebral biopsies when indicated, post mortem obtained material, tissue culture as well as biochemical determination of content and composition of basic compounds and estimation of respective enzyme activities.

Project No. Z01 NS 00706-18 DMN

Project Description:

Objectives: The majority of chronic neurological disorders make their first manifestation during childhood. Because of the chronicity and long duration of the handicaps these diseases constitute a formidable medical and social problem of our times. Pooled together, mental retardation (frequently familial) birth defects, cerebral degenerations and inborn errors of metabolism affecting the nervous system amount to over four million people in the U.S.A. Our main objectives are: 1) to study the pathogenesis and etiology of these diverse disorders which are frequently of genetic origin, 2) to devise special diagnostic tests including identification of heterozygotes, 3) to institute therapeutic modifications of the course of the respective disorders, 4) explore preventive measures such as eugenics and prenatal diagnosis.

Patient Material: Total of 22 inpatients and 36 outpatients were studied. Patients with the following disease categories were admitted for investigation in this order of frequency: mucopolysaccharidoses, sphingolipidoses, ceroid lipofuscinosis, various somatic hereditary syndromes and familial mental retardation.

Methods Employed:

1) Extended neurological, developmental and genetic assessment of the patients studied, including family study when appropriate.
2) Determination of profiles of lipids, amino acids, proteins, mucopolysaccharides and carbohydrates in blood, and when appropriate, in urine and cerebrospinal fluid.
3) Assay of enzyme activities in the peripheral blood leukocytes of genetic diseases studied; also, preparation of the karyotypes using banding technique.
4) Establishment of skin fibroblast tissue cultures in patients with genetic disorders for study of enzyme activity and turnover studies using radioactive substances. In the case of certain sphingolipidoses and mucopolysaccharidoses, radioactivily labelled substrates or elements are added to respective tissue cultures. In the first instance the catabolism, in the second, synthesis of the respective involved substances are followed.
5) Employment of invasive techniques, if required, for definitive diagnosis. Brain and liver biopsies are performed and the tissues are used for biochemical, chemical, enzymatic and electron microscopic studies.
6) Therapeutic modification of the diseases is attempted whenever possible. For this purpose we use pharmaceuticals, hormones, plasma or formed blood elements transfusion, dietary modifications and others.
7) In case the patient with a metabolic disease dies, samples of the organs and other tissues are stored frozen for future chemical and enzymatic studies; the fresh specimens of tissue are immediately fixed or processed for histochemical and electron microscopic studies.

Major Findings:

For the past 10 years a considerable portion of our efforts and resources were directed to elucidation of pathogenesis of mucopolysaccharidoses. This has rendered several substantial contributions. The most important are in two areas: one - determination of the content and composition of mucopolysaccharides in the body fluids and tissues of patients with different types of this disorder; two - demonstration of diverse ultrastructures in the brains and certain organs of these patients. The material was obtained by biopsy or at autospy.

1) Multidisciplinary studies were conducted on the brain and other tissues of patients who died with the antemortem diagnosis of mucopolysaccharidosis (MPS) of one of the following types: Type V, Scheie disease (MPS-V); type I, Hurler disease (MPS-I); and type II, Hunter disease (MPS-II). The principal new finding in the brain of the patient with MPS-V is the presence of lesions in the periadventitial mesenchymal tissue of the white matter, similar to those of MPS-I, while the nerve cells in MPS-V are histologically normal, in contradistinction to MPS-I, in which the neuronal abnormality is severe. Electron microscopical studies of the brain in MPS-I demonstrated numerous complex membranous inclusions in the neurons, whereas the neurons in MPS-V contained only a small number of lipofuscin-like inclusions and typical lipofuscin granules. There was a threefold increase of glycosaminoglycans (GAG) in the brain of MPS-I, but only a slight increase in the MPS-V; GAG in the liver and spleen of all patients was noticeably increased. α-L-iduronidase activity was not detectable in the brain and liver of patients with MPS-I and MPS-V, thus suggesting a similar enzymatic defect.

2) Histochemical and electron microscopic studies of the brains inclusive leptomeninges with large blood vessels from 7 patients with MPS I, II, IIIA and V showed marked increase in mesenchymal elements and generalized presence of characteristic lesions around cerebral veins and arteries. The periadventitial space was greatly distended and filled with viscous fluid and numerous mononuclear cells containing large cytoplasmic vacuoles; these cells stained positively for glycosaminoglycans (GAG). In contrast, the neurons showed only a slight increase of GAG over the normal controls but contained an excessive amount of glycolipid-like material. The amount of GAG in the leptomeninges inclusive large blood vessels was 10.8, 6.5, 4.5 and 2.2 times greater in patients with MPS I, II, V and IIIA respectively, than the mean from the unaffected controls. Dermatan sulfate (DS) accounted for most of the GAG increase in MPS-I, II and V (mixed excretors of DS and HS), and HS in MPS-IIIA (HS excretor). We conclude that the mesenchymal elements contribute substantially to the content of GAG, as dermatan sulfate, in the combined brain tissue.

3) Therapeutic modification studies were conducted in selected patients with MPS type III, V and VI, using long-range administration of corticosteroids (alternate day dosages). The corticosteroid administration was associated with improvement of joint mobility and temporary slight amelioration of mental performance and behavior. The metabolic changes in mucopolysaccharides was evaluated by determination of polymeric and break-down products of urinary mucopolysaccharides accompanied administration of corticosteroids. In general, most of these patients showed a short lasting initial increase in the urinary mucopolysaccharides which was then followed by a sustained reduction. Also, variable changes in ratios of dermatan sulfate to heparan sulfate took place in the patients with MPS types II and V. These results are now ready for submission to a medical journal.

4) Concentrated efforts were made to introduce therapeutic modifications of selected inborn errors of metabolism. A measure of success was obtained in treatment of kinky hair disease with a new method of parenteral administration of copper, and guiding of chelating dosages of penicillamine of Wilson's disease by repeated metabolic balance studies of copper when both low copper diet and penicillamine were used. These results are described in the listed publications. The intravenous infusion of isolated enzymes: trihexosidase and glucocerebrosidase to the patients with Fabry's and Gaucher's disease and their effects on the excessively stored material in various tissues are described in appropriate publications.

5) Summary of studies on pathogenesis: In Wilson's disease the metabolic problem is excessive accumulation of copper in the body, probably due to inadequate excretion of copper in the bile. In Menkes' disease, on the other hand, the problem is one of copper-deficiency and the primary pathologic lesion, abiotrophy of the elastic tissue of blood-vessels, is attributable to deficiency of copper which is essential for the biosynthesis of elastin. Tracer studies of the physiology of copper in both diseases, using ^{67}Cu show significant similarities, prolonged retention in the whole-body and in the liver and decreased gastro-intestinal excretion, but also reveal the essential difference, namely, deficient absorption in Menkes' disease, normal or increased absorption in Wilson's disease.

Significance to Bio-Medical Research and the Program of the Institute:

Because the majority of infections affecting man are now under quite satisfactory control, the time has come for increased attention to accord a measure of control to such common disorders as hereditary diseases, congenital malformations, mental retardation and degenerative conditions affecting the nervous system. Improved methodology makes it now feasible to advance our knowledge and institute some control in certain of these crippling chronic disorders. Prevention and therapy include prenatal diagnosis, enzyme infusions, dietary modifications and institution of certain eugenic measures. Since many of the disorders affect exclusively or predominantly the nervous system the study of etiology and pathogenesis as well as institution of therapeutic trials are of importance in furthering the main mission of our Institute.

Proposed Course of the Project: Clearly this is a broad and long range project of our Section. During the next years increasing emphasis will be given to the underlying genetic mechanisms of the hereditary diseases and to therapeutic modifications of respective disorders.

Publications:

1. Dekaban, A.S., and Brady, R.O.: Therapeutic approaches to selected disorders of inborn errors of metabolism with neurological involvement. Int. J. Neurology, 11: 46-61, 1976.

2. Dekaban, A.S.: Inborn errors of copper metabolism: Kinky hair disease and hepatolenticular degeneration. Materia Medica Polna, 2 (27): 1-6, 1976.

3. Whyte, M.P., and Dekaban, A.S.: Familial cerebral degeneration with slow eye movements, mental retardation and incidental nevus of Ota. Child Neurol. and Developm. Med., 18: 373-380, 1976.

4. Constantopoulos, G., McComb, R.D., and Dekaban, A.S.: Neurochemistry of the mucopolysaccharidoses: Brain glycosaminoglycans in normals and four types of mucopolysaccharidoses. J. of Neurochem., 26: 901-908, 1976.

5. Dekaban, A.S., Constantopoulos, G., Herman, M.M., and Steusing, J.K.: Mucopolysaccharidosis Type V (Scheie Syndrom). A postmortem study by multidisciplinary techniques with emphasis on the brain. Arch. Pathol., 100: 237-245, 1976.

6. O'Reilly, S., Dekaban, A.S., Aamodt, R., Rumble, W.F., and Johnston, G.S.: Wilson's disease and Menkes' disease - A contrast in chronogenetics. Acta Med. Auxol., 8: 147-154, 1976.

7. Dekaban, A.S., and Constantopoulos, G.: Mucopolysaccharidosis types I, II, IIIA and V: Pathological and biochemical abnormalities in the neural and mesenchymal elements of the brain. Acta Neuropath. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 00815-17 DMN

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism of Complex Lipids of Nervous Tissue.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. O. Brady, Chief, Dev. & Metab. Neurol. Br.,	DMN, NINCDS
	P. G. Pentchev, Biochemist	DMN, NINCDS
	A. E. Gal, Organic Chemist	DMN, NINCDS
	J. W. Kusiak, Staff Fellow	DMN, NINCDS
	F. S. Furbish, Staff Fellow	DMN, NINCDS
	J. A. Barranger, Clinical Associate	DMN, NINCDS

COOPERATING UNITS (if any)

Weizmann Institute of Science, Rehovot, Israel
Tufts University Medical School, Boston, Massachusetts

LAB/BRANCH

Developmental & Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.1	5.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Procedures are developed for the purification of enzymes from tissues such as human placenta that are lacking in patients with Gaucher's disease, Fabry's disease, Tay-Sachs disease and Niemann-Pick disease and mannosidosis. The effects of enzyme replacement therapy in patients with these disorders is under investigation. Procedures are developed for the diagnosis of patients with these disorders, the detection of heterozygous carriers of these genetic traits, and for the detection of heterozygous carriers of these genetic traits, and for the monitoring of pregnancies at risk for each of these diseases.

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebrosides, gangliosides, and sphingomyelin: (2) to study the control mechanisms which regulate these processes; (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states, and (4) to provide diagnostic and therapeutic procedures for the amelioration and control of the lipid storage diseases.

Methods: Glucocerebroside and galactocerebroside labeled with ^{14}C in either the hexose or fatty acid portion of the molecule have been synthesized. ^{14}C-labeled sphingomyelin and gluco-and galactopsychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetra hexoside (globoside) specifically labeled with radioactive hydrogen-^{3}H have been prepared. The metabolism of these labeled materials has been investigated in vivo and in vitro. Human placenta has proved to be a convenient and rich source of sphingolipid hydrolases. Isolation of these enzymes for therapeutic replacement trials is a major continuing portion of this project.

Major Findings: (1) We have previously reported that enzyme replacement in Gaucher's disease and Fabry's disease promises to be effective therapeutic procedure for the amelioration of these disorders. A long lasting reduction of blood glucocerebroside, the accumulating lipid in Gaucher's disease,was observed in two of three patients infused with purified human placental glucocerebrosidase. Patients with Gaucher's disease have subnormal activity of this enzyme in their tissues. The lack of change in blood glucocerebroside in the third recipient is most likely due to the fact that only 8% of the accumulated lipid was cleared from the liver in this patient whereas there was a 26% reduction in accumulated in liver glucocerebroside in the first two patients. The discrepancy in the percentage cleared was due to the fact that the third patient had 24 times more glucocerebroside in her liver than the first and 11 times more than the second. There was a constant amount of glucocerebroside cleared per unit of enzyme injected. Thus, we now know precisely how effective the exogenous glucocerebrosidase is in vivo. Therefore, we can estimate how much enzyme will be required by Gaucher patients by determining the amount of glucocerebroside in a liver biopsy specimen obtained prior to the enzyme replacement trial. We have devised a method for the purification of glucocerebrosidase on a large scale in a form that is suitable for administration to humans. Further enzyme replacement trials in Gaucher's disease are underway at this time. (2). We have found that sedation and lowering the body temperature of animals injected with human placental glucocerebrosidase dramatically prolongs the presence of the exogenous enzyme in the animal tissues. This observation has great potential importance for enzyme replacement in humans if it can be shown that the exogenous enzyme is catalytically active over the extended period of time. We are currently devising experiments to provide this information.

(3). We have developed a method for the purification of sphingomyelinase, the enzyme lacking in Niemann-Pick disease, also from human placental tissue. At the present time, it is very difficult to obtain sufficiently large quantities of this enzyme for replacement therapy trials in humans with Niemann-Pick disease. However, during the past year, we discovered that the administration of the compound known as AY-9944 to rats causes a dramatic reduction in the amount of sphingomyelinase in the liver and other organs in the recipients. The reduction in enzymatic activity is accompanied by an increase in tissue sphingomyelin, and Niemann-Pick like inclusion bodies appear in retinal neurons, an important pathological correlate of the disease in humans. AY-9944 does not affect the catalytic activity of the enzyme. The reduction of sphingomyelinase activity in the animal model is specific. It is caused by a decrease in the production of this enzyme. Thus, the pharmacologically induced animal model has all of the requirements for an examination of the effect of exogenous sphingomyelinase on the biochemical and pathological consequences attending this enzyme deficiency. We propose to use this animal model to explore the effect of enzyme replacement therapy in Niemann-Pick disease.

(4). Since our original trial of enzyme replacement in patients with Tay-Sachs disease in 1972, it has been apparent that such individuals would not be benefited by the intravenous administration of the required enzyme without additional measures. This constraint is imposed by the blood-brain barrier which prevents the entrance of molecules as large as enzymes into the central nervous system. In order to examine the feasibility of temporarily opening the barrier, we carried out a survey of lysosomal hydrolases in brain to determine if one of these might exhibit an appropriate level of activity so that superposition of exogenous enzyme would be readily and reliably detected over the basal enzyme activity. It was discovered that mannosidase was a likely candidate for such an investigation. Furthermore, individuals with insufficient mannosidase activity are known to be severely retarded mentally, so there is also a valid clinical rational for such an undertaking. The requisite mannosidase was purified from human placental tissue. The blood-brain barrier was opened temporarily by intracarotid infusion of hypertonic solutions of mannitol or arabinose. Under these experimental conditions, a clear augmentation of mannosidase activity occurred in the brain after intravenous injection of the enzyme. This increase in brain mannosidase was fully equivalent to the physiological level of this enzyme. This first demonstration of the accessibility of exogenous enzyme to the central nervous system is a major accomplishment towards enzyme replacement therapy for the multiplicity of heritable disorders that cause brain damage.

(5) We continue to serve as a center for the diagnosis of patients and detection of carriers for all of the lipid storage diseases. Much effort is devoted to the monitoring of pregnancies at risk for heritable metabolic disorders. During the past year, we performed more than 270 diagnostic assays for physicians and genetic counselors from all over the world.

Significance: Enzyme replacement appears to offer considerable promise for the treatment of Gaucher's disease and Fabry's disease. It is expected that the deleterious clinical course in these patients will be ameliorated by this therapy. The newly developed ability to introduce enzymes into the central nervous system has profound implications for the treatment of genetic disorders that cause brain damage.

Proposed Course: We will continue to carry out and monitor the long-term effects of enzyme infusion in patients with Gaucher's disease, Fabry's disease, and mannosidosis. Studies of enzyme replacement with purified sphingomyelinase will be initiated in the newly developed animal model of Niemann-Pick disease. We shall attempt to design a system to incorporate exogenous enzymes in a metabolically useful fashion.

Publications:

1. Brady, R. O.: Inherited metabolic diseases of the nervous system. *Science* 193: 733-739, 1976.

2. Brady, R. O.: Sphingolipidoses and other lipid metabolic disorders. In Siegel, G. J., Albers, R. W., Katzman, R. and Agranoff, B. W. (Eds.): *Basic Neurochemistry, Second Edition*. Boston, Little, Brown, pp. 550-568, 1976.

3. Brady, R. O.: Diagnosis and understanding of sphingolipidoses in the newborn. In Young, D. S. and Hicks, J.M. (Eds.): *The Neonate: Clinical Biochemistry, Physiology, and Pathology*. New York, John Wiley pp. 187-198, 1976.

4. Brady, R. O.: Enzymological approaches to the lipidoses. *Ann. Clin. Lab. Sci*. 7: 105-112, 1977.

5. Brady, R. O.: Heritable catabolic and anabolic disorders of lipid metabolism. *Metabol. Clin. Exp*. 26: 329-345, 1977.

6. Brady, R. O., Pentchev, P. G., Gal, A. E., Hibbert, S. R., Quirk, J. M., Mook, G. E., Kusiak, J. W., Tallman, J. F., and Dekaban, A. S.: Enzyme replacement therapy for the sphingolipidoses. In Volk, B. W. and Schneck, L. (Eds.): *Current Trends in the Sphingolipidoses and Allied Disorders*. New York, Plenum Press, pp. 523-532, 1976.

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9. Tallman, J. F. and Brady, R. O.: Disorders of ganglioside catabolism. In Rosenberg, A. and Schengrund, C.-L. (Eds.): Biological Roles of Sialic Acid. New York, Plenum Press, 1976, pp. 183-199.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01026-15 DMN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Treatment of Epilepsy--Clinical and Biochemical Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Anatole S. Dekaban, M.D.,Ph.D.	Associate Chief	DMNB	NINCDS
Other:	Michael P. Whyte, M.D.	Clinical Associate	DMNB	NINCDS
	Daniel E. Britton, M.D.	Clinical Associate	DMNB	NINCDS
	Jan K. Steusing	Research Assistant	DMNB	NINCDS

COOPERATING UNITS (if any)
Department of Psychology, University of Washington, (in respect to performance testing only)

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.9	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Epilepsy is a common neurological disorder often resulting in considerable disability over many years of a patients life. The most effective treatment in the majority of patients is the use of various anticonvulsant agents. There is marked variablity of the metabolism of the anticonvulsants from patient to patient. A dosage regimen considered therapeutic may result in considerable slowing of mental performance. Our investigative work includes: 1) Clinical determination of the most effective therapeutic regimen, 2) Chemical determination of plasma levels of the anticonvulsants and their metabolites, 3) Turnover studies of these medications and their metabolites utilizing non-radioactive labelled anticonvulsants, 4) Use of special testing procedures to determine changes in patients mental performance with variation of their therapeutic regimen.

Project Description:

Objectives: Diagnosis, therapy and long-range management of epileptics, especially during childhood has been investigated and advanced by this Section during the past years. The first of our current studies is centered on the assessment of the metabolism of anticonvulsant drugs in patients whose seizures are particularly difficult to control. The second study relates to the management of both children and adults with chronic epilepsy in prevention of excessive drowsiness and slowing of mental activity resulting from high doses of anticonvulsant medication. Since no satisfactory tests for subtile variations in the mental performance in epileptics are available, we have decided to ameliorate this situation.

Patient Material: (41) inpatients and (13) outpatients.

Methods Employed:

1. Several categories of patients were studied: a) those whose epileptic seizures are difficult to control, both children and adults, b) those patients with seizures who in addition have endocrinopathy, c) those patients with seizures who in addition to anticonvulsants are receiving various hormonal preparations.
2. Using gas-liquid chromatography serum, cerebrospinal fluid and urine profile of anticonvulsants and their metabolites were determined according to our procedure and methods published in Clin. Neurol. and Neurosurg., 3/4: 168-179, 1974.
3. Using deuterium labelled phenobarbital, its fate in the body and metabolites is studied.
4. Clinical methods inclusive daily observation of seizures are used for determination of the efficacy of medication.
5. Especially devised psychological performance tests are used to monitor the effects of various anticonvulsants on mental performance.

Major Findings:

1. Biotransformation of phenobarbital (PB) to p-hydroxyphenobarbital (PHPB) was studied quantitatively by gas-liquid chromatography in 8 epileptic patients who were receiving an estabished regimen of antiepileptic drugs including PB. PB and both conjugated and unconjugated PHPB were present in each patient's urine, m-hydroxyphenobrabital (MHPB) was not detected despite an assay sensitivity of 0.25 μg/ml. Incubation of the urine with β-glucuronidase bur not with arylsulfatase liberated PHPB which was therefore presumed to be conjugated with glucuronic acid. In general, the patients urine contained more PB than total PHPB. Recovery of the patient's total daily dose of PB ranged from 24 to 77% (mean, 42%). After receiving a single dose of PB intravenously, PB and both conjugated and unconjugated PHPB were found in a normal volunteer's

urine throughout a 16-day collection period; 30% of the dose was recovered. PB excretion was proportional to urine volume in the volunteer and in two additional patients who were made to vary their daily fluid intake. PHPB was not detected in the cerebrospinal fluid of 10 patients receiving PB. Neither PB, PHPB nor MHPB were detected in the feces of four patients. These results suggest that metabolites other than PHPB or MHPB may be important in the elimination of PB in man.

2. Appropriately modified cognitive tests were applied during three consecutive days to 19 patients with epilepsy, first under medium therapeutic dose of phenobarbital (Week 1) and then under high therapeutic dose (Week 2) of this drug. The following finding is both unexpected and important: short-term memory scanning rate was strikingly slower during Week 2 ($p < 0.001$) when the patients were on the increased dose of phenobarbital, while retrieval of information from the long-term memory was unaffected. Twenty normal controls without medication were tested in a similar manner and did not show significant effects of practice.
3. Several recent reports in the literature suggested presence of disturbance in acid mucopolysaccharides (GAG for glycosaminoglycans) metabolism in the patients with familial myoclonus epilepsy. We have conducted a comprehensive study on the urinary GAG in five patients with myoclonus epilepsy and their parents and did not detect a significant alteration in the content or composition of this compound. Thus, it is not probable that the disturbance of mucopolysaccharides underlies metabolic abnormality in myoclonus epilepsy.

Significance to Bio-Medical Research and the Program of the Institute:

Over two million people in this country suffer from epilepsy. The life of affected patients and their families is greatly handicapped and there is an enormous economic loss. Currently, the time has come to place treatment of epilepsy on a scientific basis using biochemical parameters and performance tests to avoid unnecessary mental impairment caused by higher doses of medication.

Proposed Course of the Project: The modification of treatment of recurrent cerebral seizures and a better control of the type and dosages of the medication used by determination of balance studies of antiepileptic drugs will continue. Simultaneously, we will proceed with the development of suitable methods for mental performance testing which could be used in ambulatory settings.

Publications:

1. Whyte, M.P., and Dekaban, A.S.: Metabolic fate of phencbarbital: A quantitative study of p-hydroxyphenobarbital elimination in man. Drug Metabolism and Disposition, 5: 63-70, 1977.

2. Constantopoulos, G., Dekaban, A.S., Steusing, J., and Eldridge, R.: Content and composition of urinary glycosaminoglycans in the patients with progressive myoclonus epilepsy with and without Lafora bodies. Acta Neurologica Scand. (in press).

3. MacLeod, C., Dekaban, A.S., and Hunt, E.: Selective memory impairment in epileptic patients on phenobarbital. Science (in press).

ITHSONIAN SCIENCE INFORMATION EXCHANGE OJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01309-12 DMN

ERIOD COVERED
July 1, 1976 through September 30, 1977

ITLE OF PROJECT (80 characters or less)
Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

AMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Fishman, Ph.D., Research Biochemist,	DMN, IRP, NINCDS
OTHER:	R. O. Brady, M.D., Chief,	DMN, IRP, NINCDS
	T. Pacuszka, Ph.D., Visiting Fellow,	DMN, IRP, NINCDS

COOPERATING UNITS (if any)
Lab. Cell Metabolism & Mol. Dis. Branch, NHLBI; Lab. Biochem. Pharm., NIAMDDS; Dept. of Immunol., WRAIR

LAB/BRANCH
Enzymology and Genetics

SECTION
Developmental & Metabolic Neurology Branch

INSTITUTE AND LOCATION
NINCDS, IRP, Bethesda, Maryland 20014

TOTAL MANYEARS: 3.1	PROFESSIONAL: 2.1	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

One function of gangliosides is to serve as biotransducers of membrane-mediated information. Ganglioside G_{M1} is the surface membrane receptor for cholera toxin. The toxin, which is multivalent, binds to the oligosaccharide chain of G_{M1} via the terminal sialyl and galactosyl residues. Binding induces shifts in the fluorescence and circular dichroic spectra of the toxin indicative of a conformational change in the protein. Binding of cholera toxin to lipid model membranes (liposomes) containing G_{M1} induces a perturbation in the membrane structure. These changes in the toxin and membrane structures presumably are involved in the mechanism whereby cholera toxin activates membrane-bound adenylate cyclase.

Gangliosides also interact with glycoprotein hormones such as gonadotropin and thyrotropin. Thyroid membranes contain a complex pattern of over 30 different gangliosides which are synthesized by a family of glycosyltransferases localized in the Golgi apparatus. Several of these thyroid gangliosides inhibit the binding of thyrotropin to the thyroid membranes; the most potent inhibitor is present in only trace amounts and appears to have a novel structure. Thyroid gangliosides may combine with membrane glycoproteins to form a functional thyrotropin receptor on the thyroid cell.

Project Description:

Objectives: To investigate the function of membrane glycosphingolipids in the regulation of cell proliferation, cell morphology, hormone action and toxin sensitivity; to explore the regulation of glycosphingolipid biosynthesis during development and differentiation and relate these findings to anabolic heritable disorders; to determine the underlying mechanism of altered glycosphingolipid biosynthesis in neoplastic tissues; these studies are being extended to other membrane glycoconjugates.

Methods: The glycosphingolipid composition of cultured cells and tissues is determined by extraction and purification of this class of lipids followed by separation of individual glycolipids on thin-layer chromatograms. Metabolism in cultured cells is determined by adding radiolabelled sugars to the culture medium and isolating the labelled glycosphingolipids. Biosynthesis in vitro is analyzed by assaying the activities of the glycosyltransferases involved in glycosphingolipid synthesis. Surface glycoconjugates are labelled by selective oxidation with galactose oxidase or periodate and subsequent reduction with sodium borotritide. Gangliosides radiolabeled in specific portions of the molecule are prepared by specific enzymatic reactions. Thus [^{14}C]-N-acetylneuraminyl-lactosylceramide ([^{14}C]-G_{M3}) is synthesized from lactosylceramide and CMP-[^{14}C] sialic acid with the specific sialyltransferase activity.

Binding of toxins and hormones to cells, cell membranes and liposomes is determined with [^{125}I]-labeled proteins and either filtration or centrifugation techniques. Levels of cyclic AMP and adenylate cyclase activity are measured with a modified cyclic AMP protein binding assay.

Oligosaccharides are prepared from gangliosides by ozonolysis and alkaline fragmentation. The oligosaccharides are purified by exchange and gel filtration chromatography. Purity and composition is assessed by thin layer and gas liquid chromatography.

Major Findings:

A. Interaction of Cholera Toxin with Gangliosides

1) Continuing our studies on the role of ganglioside G_{M1} as the cholera toxin receptor on cell membranes, we observed that serum which contains gangliosides sensitized G_{M1}-deficient cholera toxin-insensitive mouse cells when the cells were grown in medium supplemented with calf serum. The cells took up small amounts of G_{M1} ($\sim$80,000 molecules/cell) from serum which was sufficient to allow the toxin to activate adenylate cyclase in the cells. This observation has important implications for the extracellular circulation of gangliosides which appear to be bound to serum

2) Cultured human fibroblasts contain small amounts of complex angliosides including G_{M1}, which they are able to synthesize. hese cells are very sensitive to cholera toxin. Using surface abeling techniques, we demonstrated a direct interaction between holera toxin and endogenous G_{M1} receptors on the cell surface (Moss, anganiello and Fishman, Biochemistry 16: 1876-1881, 1977). Cholera oxin protected the terminal sialyl and galactosyl residues of G_{M1} from eing labeled; other surface glycolipids were not protected.

3) Cholera toxin bound to liposomes containing G_{M1} and induced he release of trapped glucose from the liposomes (Moss, Fishman, ichards, Alving, Vaughan and Brady, Proc. Nat. Acad Sci. USA 73: 480-3483, 1976). This binding toxin to G_{M1} receptors on the liposome nduced a change in membrane permeability. Cholera toxin is composed of A nd B protomers. The A protomer is involved in the activation of denylate cyclase and B in binding of the toxin to G_{M1} receptors. The protomer was as effective as toxin in causing glucose release from $_{M2}$-liposomes; only the A protomer, however, was able to bind to $_{M1}$-free liposomes (Moss, Richards, Alving and Fishman, J. Biol. Chem. 52: 797-798, 1977). Thus, the A protomer appears to be shielded in the ntact toxin but, once freed of the B, can bind to liposomes.

Cholera toxin bound slightly to liposomes containing G_{M2} or $_{A1}$, partially protected the glycolipids from galactose oxidase-aB[^{3}H]$_4$ labeling, but did not cause the release of trapped glucose hese glycolipids are similar to G_{M1} except for the absence of the erminal galactose or sialic acid residues. A specific perturbation of he toxin molecule (presumably by binding to the oligosaccharide chain f G_{M1}) is required for the release of trapped glucose from liposomes. hese studies provide a useful model for examining the interaction of holera toxin with membranes.

4) The oligosaccharide moieties of G_{M1} and other gangliosides ere prepared. Oligo-G_{M1} induced a blue-shift in the fluorescence pectra of cholera toxin and its B protomer similar to that observed ith G_{M1}; other oligosaccharides did not cause this shift. Oligo-$_{M1}$ also caused a decrease in the circular dichroic (CD) spectra of holera toxin and its B protomer. Maximal changes in fluorescence and D spectra were observed when the molar ratio of oligo-G_{M1} to toxin as between 5 and 6 to 1. Thus, binding of the B protomer of cholera oxin to the oligosaccharide chain of its receptor appeared to induce conformational change in the toxin molecule which is multivalent. irect conformation of multiple binding sites was obtained by equilirium dialysis and gel filtration chromotography experiments.

Interaction of Glycoprotein Hormones with Gangliosides

1) Gangliosides inhibited the binding of human chorionic onadotropin (hCG) to testicular cell membranes and induced changes n the fluorescence spectrum of dansylated hCG (Lee, Aloj, Brady and

Kohn, Biochem. Biophys. Res. Commun. 73: 370-377, 1976). Thus, gangliosides can interact with hCG analogous to their interaction with thyrotropin (TSH). The specificity of the gangliosides, however, was different; thus, with hCG, G_{D1a} was a better inhibitor than G_{D1b} whereas the reverse was found with thyrotropin.

2). Membranes from rat thyroid tumor were missing complex gangliosides and the requisite glycosyltransferase activities when compared to normal rat thyroid membranes (Meldolesi, Fishman, Aloj, Kohn and Brady, Proc. Nat. Acad. Sci. USA 73: 4060-4064, 1976). We were unable to label any gangliosides when the tumor membranes were exposed to galactose oxidase and NaB^3H_4; gangliosides from normal membranes were labeled. These tumor cells are unable to bind TSH and do not respond to the hormone. Thus, thyroid membrane gangliosides may be involved in the transmission of the hormonal message to the interior of the thyroid cell.

3). Bovine thyroid glands were found to contain over 30 different gangliosides. Thyroid glands have the glycosyltransferase activities necessary for the step-wise synthesis of gangliosides and these enzymes are localized in the Golgi apparatus. We were able to identify the individual reactions for the synthesis in vitro of G_{D1a} and G_{T1} from lactosylceramide.

4) Several of the purified thyroid gangliosides inhibited the binding of TSH to thyroid membranes. The most potent inhibitor was present in trace amounts ($\sim$10,000 molecules per thyroid cell) and appeared to have a novel structure with at least two sialic acids.

5) When bovine thyroid membranes were treated with detergent, most of the TSH binding activity was solubilized and associated with membrane glycoproteins, whereas most of the gangliosides remained in the insoluble fraction (Meldolesi, Fishman, Aloj, Ledley, et al, Biochem. Biophys. Res. Commun. 75: 581-588, 1977). Extraction of the membranes with chloroform-methanol solvent reduced TSH binding by $\sim$70%. Binding properties of the solubilized fraction and the delipidated membranes, however, were different from intact membranes. Thus, specific thyroid gangliosides may combine with membrane glycoproteins to form a functional thyrotropin receptor on the thyroid cells.

Significance: These studies are providing information of the function of membrane glycosphingolipids. Gangliosides appear to play a role in the transmission of information across cell membranes. This function has directly been demonstrated for cholera toxin and is implied for certain glycoprotein hormones such as TSH and hCG. Ganglioside receptors may differ from classical receptors in that they promote a perturbation of the protein ligand that leads to a functional effect (i.e. changes in membrane permeability and activation of membrane-bound enzymes).

Proposed Course: The project will be continued with emphasis placed on the potential role of gangliosides as receptors for other proteins. We will continue to examine various clinical disorders for possible abnormalities in ganglioside biosynthesis especially disorders of the thyroid and gonads.

Publications:

1. Brady, R. O. and Fishman, P. H.: Alterations of Galactosaminyl and Galactosyl Transferases in Cultured Mammalian Cells and In Vivo. In Martonosi, A. (Ed.): Membrane-Bound Enzymes. New York, Plenum Press, 1976, pp. 421-442.

2. Fishman, P. H. and Brady, R. O.: The Altered Metabolism of Sialic Acid Containing Compounds in Tumorigenic Virus-Transformed Cells. In Rosenberg, A., and Schengrund, C.-L. (Eds.): Biological Roles of Sialic Acid. New York, Plenum Press, 1976, pp. 239-274.

3. Fishman, P. H. and Brady, R. O.: Biosynthesis and Function of Gangliosides. Science. 194: 906-915, 1976.

4. Fishman, P. H., Moss, J., Vaughan, M.: Uptake and Metabolism of Gangliosides in Transformed Mouse Fibroblasts: Relationship of Ganglioside Structure to Choleragen Response. J. Biol. Chem. 251: 4490-4494, 1976.

5. Fishman, P. H., Moss, J., and Manganiello, V. C.: Synthesis and uptake of gangliosides by choleragen-responsive human fibroblasts. Biochemistry 16: 1871-1875.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01457-11 DMN

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Chemical Synthesis of Radioactive Sphingolipids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. E. Gal, Sect. Head, Research Chemist, DMN, NINCDS
OTHER: F. J. Fash, Bio. Lab. Technician, DMN, NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Neurochemical Methodology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Radioactive isotopes were synthesized and used for metabolic studies and as diagnostic tools in sphingolipidoses. The labelings were made by ^{14}C and tritium by gas exposure, synthetic and semi-synthetic techniques and a new approach: Functional group exchange.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To prepare sphingolipids labeled with radioactive isotopes. The compounds are used for metabolic studies and as diagnostic tools in investigations related to hereditary lipid storage diseases.

Methods and Major Findings: A multitude of approaches were used in labelling glycolipids such as chemical synthesis, partial synthesis, minor synthetic modifications, functional group exchange and tritium gas exposure. These methods could be classified into two categories: specific and non-specific labelling. The ideal approach is the specific labelling which consist of the tagging of a complex molecule at a predetermined atom. Total synthesis is the best way to accomplish this but up to now only few sphingolipids have been synthesized. We synthesized sphingosine, psychosine and galactocerebroside specifically labelled by total synthesis. However, our main effort is directed toward methods which would allow specific labelling of atoms yet would not necessitate tedious syntheses. An interesting technique which we developed is called the functional group exchange. A chemical group such as an acetyl or carboxyl is split from a molecule and is replaced with a similar but radioactive one. With this approach we could prepare aminosugars even gangliosides. Using the approach-minor synthetic modification; we prepared asialo ganglioside, Tay Sachs ganglioside and ceramidetrihexoside. In this approach oxidation and reduction of an alcohol group in the molecule with a radioactive reducing agent would reestablish the original lipid in radioactive form. The lipids used as starting material for this approach were isolated from human tissues. Tritium gas exposure a non-specific approach, was repeatedly used for labelling ceramide dihexoside, dihexoside and globoside. By this method all the non-labile hydrogen atoms in a molecule become radioactive. This procedure is relatively simple but the purification of the resulting compounds are complex. Also this type of compound require more elaborate enzyme assays.

Significance: The compounds are indispensable in the detection, identification and isolation of enzymes connected to lipid storage diseases. Also studies related to qualitative and quantitative determination of enzymes in animal or human tissues necessitate these labelled substrates. Prenatal diagnoses are of rising importance. These labelled compounds play a key role in these diagnostic procedures. As a therapeutic approach, this branch initiated replacement therapy by the administration of the missing enzyme in hereditary diseases. The monitoring of the enzyme levels during and after this therapeutic procedure was done by the use of these radioactive substrates. It would be also of great interest to develop new methods which would allow to prepare relatively easily and inexpensively these compounds for the use of clinicians and for researchers who are not connected to large research center.

Proposed Course: Work on this project continues in three major directions: 1. Glycolipids will be labeled by using the above mentioned techniques with ^{14}C and Tritium. 2. The approach using "minor synthetic modification: will be extended and used on lipids which were not prepared at all or not prepared by this technique. Also the replacement of the enzymatic oxidation will be explored. 3. Work will continue on the development of the technique: labeling of functional group exchange.

Publications:

See Project Nos. Z01 NS 00815-17 DMN and Z01 NS 00816-17 DMN.

SMITHSONIAN SCIENCE
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PERIOD COVERED
July 1, 1976
TITLE OF PROJECT
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PI:
OTHER :

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01480-10 DMN

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Metabolism of Neurohumoral Transmitter Substances in Marine Animals

LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. G. Trams, Chief, Section Physiol. & Metabolism, DMN
OTHER : A. A. Benson, Assoc. Director, Scripps Inst. Ocean.
S. Patton, Borland Prof., Div. Food Science, Penn. State Univ.
P. Gilbert, Director, Mote Marine Lab.

COOPERATING UNITS (if any)
Scripps Inst. Oceanography, LaJolla, Ca
Div. Food Sci., Penn State Univ., University Park, Pa.
Mote Marine Lab., Sarasota, Florida

LAB/BRANCH
Developmental and Metabolic Neurology Branch, DMN

SECTION
Physiology and Metabolism

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to explore the great variety and abundance of the marine environment for molecular models of neurobiology. In particular, we search for species or phenomena which display an amplification or simplification of human physiology or pathology. Typical examples of this work were studies on membranogenesis employing developing sea urchins, bioelectrogenesis in the electric eel and torpedo, and brain pathology in the migrating Pacific salmon. During the past year we have completed an investigation of brain Ca^{2+}-ATPases in several vertebrate species. There was a positive correlation of enzyme activity with the presumptive position of a species on the evolutionary scale.

Project Description:

Objectives: To exploit for the study of neurobiology the great variety and abundance and the biochemical specialization of oceanic life forms. Such models have been explored by many investigators (e.g. squid giant axon, torpedo electroplax, tetrodotoxin) and we have introduced several others during the course of this project. Of the latter, substantial interest have been shown by scientific and lay community in use of the migrating Pacific salmon as a model for the aging process and for certain degenerative diseases. Another example was the use of developing sea urchin embryos in membranogenesis.

Methods: The primary mission of the Section on Physiology and and Metabolism, is to probe into the molecular basis of bioelectrogenesis and neurochemical transmission. In the exploration of marine organisms we have therefore made primarily use of model systems which allowed a facile interrogation of plasma membranes, or provided us with fairly simple CNS structures (as in elasmobranchi), or seemed to offer an analogy to certain diseases of the central nervous system in man. The laboratory methodology is essentially classical but modified for the species under investigation. Occasionally more unusual methods were required e.g. in order to maintain a salmon heart beating for several hours on a shipboard laboratory or to obtain EEG tracings from free swimming elsmobranchi.

Major Findings: During a concurrent investigation on plasma membrane ecto-enzymes we had come to the conclusion that a major portion of brain Ca^{2+}-ATPase activity was due to ecto-ATPases. In addition it appeared likely that the biochemical lesion(s) in animal models of epilepsy was either directly or closely related to nucleotide metabolism in the brain. We therefore conducted a comparative study on brain ATPases in several vertebrate species. Part of this investigation was carried out aboard the R/V "SEVERIANA" and supported in part by the Foundation for Ocean Research, the National Science Foundation and the Scripps Institution of Oceanography. Other work was done at, and with the cooperation of the staff of, the Mote Marine Laboratory in Sarasota, Florida.

A standardized preparative procedure was developed for obtaining brain Ca^{2+}-ATPases from a variety of animal species. Of primary interest was the characterization of the different brain enzymes according to sensitivity towards stimulation by divalent cations. In other experiments typical enzymologic data were developed, including kinetic constants. Enzymes were obtained from the following: several elasmobranchi and teleosti, snakes and turtles, chicken, hamsters, rats and gerbils and human cortex.

These conclusions were drawn from observations made in this study: 1. Brain ATPase associated with membranous elements, and possessing broad substrate specificity, is common to all vertebrates. 2. The enzyme(s) required divalent cation for activity and there was a species-specific variation by which either Ca^{2+} or Mg^{2+} or Mn^{2+} were optimally activating. 3. The characteristics of the enzymes obtained from the brains of different species were similar to those in cultured brain cells. The observed similarities suggested that the major part of measured brain ATPase activity is due to plasma membrane acto-ATPases.

Proposed Course: To extend this study to primitive nervous systems, such as found in invertebrates.

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July 1, 1976

TITLE OF PROJECT
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01481-10 DMN

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on the Composition and Metabolism of Cellular Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Trams, Chief, Section Physiology and Metabolism	DMN, NINCDS
OTHER:	C. J. Lauter, Chemist	DMN, NINCDS
	L. Firouzi, Visiting Fellow	DMN, NINCDS
	A. M. De Duffard, Guest Scientist	DMN, NINCDS

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, NIMH

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Physiology and Metabolism

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to elucidate the inter-relationship between molecular composition and topographic arrangements of membrane building blocks with reference to plasma membrane functions. Bioelectrogenesis, transport and other metabolic phenomena are based on the proper function of characteristic membrane subassemblies. Plasma membranes are studied after isolation from tissues or in intact cells of CNS derived tissue cultures. In particular the function of membrane ecto-enzymes has been the objective of this study. Experiments on seizure-prone animals indicate that malfunctioning ecto-enzymes in the brain are related to the biochemistry of convulsive disorders. The biochemical lesion in animal models of idiopathic epilepsy seems to be associated with a derangement of nucleotide metabolism in certain brain areas.

Project No. Z01 NS 01481-10 DMN

Project Description:

Objectives: To elucidate what role the molecular composition and topographic arrangement of membrane building blocks play in defining the physiological functions of the plasma membrane. Furthermore, to inquire if cell pathology in certain diseases of the CNS is associated with a derangement of function of known membrane components.

Methods: One of the primary tools used in this investigation is a stock of cultured cell lines which originated from a variety of CNS cells. Established clones of murine and human neuroblastoma, gliomas as well as several primary cell cultures developed in our laboratory are employed. Primary use of these cultures is in the study of cell surface biochemistry.

A variety of animal models for idiopathic epilepsy have been employed in experiments which were designed to delineate molecular differences between seizure-prone and control animals. Mice of several strains, chicken, hamsters and grasshopper mice were used in this project.

In order to determine to what extent the protein matrix of a plasma membrane influences its lipid composition we have developed a model system in which presumptive membrane proteins can interact with membrane lipids in a lipophilic environment.

Major Findings: Substantial progress was made in the study of plasma membrane ecto-enzymes, especially those which have been of particular interest to us, namely the ecto-phosphoester hydrolases. We have been able to broaden the characterization of ecto-5'-nucleotidases and ecto-ATPases. Both of these ecto-enzymes have been found to be ubiquitous in eukaryotic cells (certain erythrocytes excepted). It was found that the ecto-ATPases display a characteristic activation pattern in respect to divalent cations and it is conceivable that cell types can be identified by this activation pattern of the ecto-ATPase. It was also observed that the age of a cell (in culture) as well as its proliferative rate is correlated to significant changes in the activity of ecto-5'-nucleotidase and ecto-ATPase. In cultured astroblasts we have observed an inverse proportionality between proliferative rate and 5'-nucleotidase activity. The enzyme appears to play a role in differentiation or cell-cell recognition (or invasiveness). We found that a retardation of growth rate, and increased differentiation, reversibly increased enzyme activity in rapidly growing cultures.

We have previously reported adefect in the Ca^{2+} stimulated ATPase activity of DBA mice which are susceptible to the induction of audiogenic seizures. In a follow-up study we have interrogated several other animal models of epilepsy for possible lesions in brain ATPases. Mice, px chicks, epi chicks, hamsters, gerbils and grasshopper mice, animals in which seizures could be induced by various methods were the experimental animals. The respective normal strains or individuals served as controls. The activities of ATPases stimulated by Ca^{2+}, Mn^{2+} and Mg^{2+} and the Na^+, K^+, Mg^{2+}-ATPases were compared to the following presumptive control enzymes: 5'-nucleotidase, ADPase and p-nitrophenylphosphatase. There was a significant deficiency in Ca^{2+}-stimulated ATPase in DBA mice as compared to C57 control mice. Although we found no apparent differences in the ATPases between normal and epileptic animals in the other models, a discriminant analysis program classified all epileptic animals (except grasshopper mice) 100% correctly on the basis of our enzyme assays, Grasshopper mice were classified 80% correctly. From these observations we have concluded that a metabolic lesion existed in the seizure-prone animals which was associated with nucleotide metabolism, but was not necessarily confined to a lesion in the Ca^{2+}-ATPase system. Further studies are planned in which it will be determined if such enzymatic lesions are confined to specific anatomical regions of the brain.

In a collaborative study with the Clinical Psychobiology Branch, NIMH, we have addressed ourselves to the question of enzymatic correlates to differentiation and growth rates. It was found that a neuron specific protein (NSP), which has recently been identified as an enolase, is markedly depleted in human or murine neuroblastoma cells. The deficit is compensated for by an unspecific or glia-specific enolase. Retardation of growth rate and induction of morphological differentiation by addition of dibutyryl-cAMP to cultured neuroblastomas led to an increase in NSP. Further studies are in progress which are designed to elucidate this interrelationship.

We have over a number of years devised experiments with the aim to obtain a better description of membranogenesis. At the present time it is not understood how the composition of particular membrane matrices (e.g. ion translocators, receptors, etc.) is determined or how the membrane is assembled in toto. We have postulated that characteristic membrane proteins, of which 5'-nucleotidase adenylcylase and ecto-ATPase are classic examples, serve as templates or encoders for the required lipid composition in the matrices. In recent model experiments we have been able to effect the transfer of proteins into a lipophilic environment which contained characteristic complements of membrane lipids. It was found that the transfer of proteins into the hydrophobic phase was dependent on the lipid concentration in the organic phase. Preliminary data suggested that specific proteins associate with certain lipids. We have applied the term collocation to this selective process. Further experiments are in progress to define how the collocative properties of membrane proteins operate in plasma membrane biosynthesis.

Proposed Course: The intention is to pursue the different aspects of this project in accordance with the most promising leads which have evolved. Priority will be given to fundamental studies on the function of biocatalysts at the cell surface. Further research on animal models of epilepsy is planned, in particular, experiments with which a common denominator for convulsive tendencies can be elaborated. For the study of the collocative properties of membrane proteins it is visualized that substantial methodological work will precede a definitive description of the biochemistry of membranogenesis.

Publications:

1. Trams, E. G., Lauter, C. J. and Banfield, W. G.: On the activation of plasma membrane ecto-enzymes by treatment with neuraminidase. J. Neurochem. 27: 1035-1042, 1976.

2. Rosenblatt, D. E., Lauter, C. J. and Trams, E. G.: Deficiency of a Ca^{2+}-ATPase in brains of seizure prone mice. J. Neurochem. 27: 1299-1304, 1976.

3. Rosenblatt, D. E., Lauter, C. J., Baird, H. R. and Trams, E. G.: ATPases in animal models of epilepsy. J. Molecular Med. (in press)

4. Trams, E. G.: On the asymmetric composition of plasma membranes. In Bazan, N. and Brenner, R. (Eds.) Function and Biosynthesis of Lipids. New York, Academic Press, 1977 (in press).

5. Duffard, R. O., Fishman, P. H., Bradley, R. M., Lauter, C. J., Brady, R. O. and Trams, E. G.: Ganglioside composition and biosynthesis in cultured cells derived from CNS. J. Neurochem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01808-08 DMN

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Glycoproteins of Myelin in Development and Disease

LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. H. Quarles, Research Chemist	DMN, NINCDS
OTHER:	R. O. Brady, Chief, DMN	DMN, NINCDS
	L. J. McIntyre, Visiting Fellow	DMN, NINCDS
	R. J. McIntyre, Visiting Fellow	DMN, NINCDS
	R. N. Nishimura, Guest Worker	DMN, NINCDS

COOPERATING UNITS (if any)

Section on Cellular Neuropathology, LNNS, NINCDS, NIH
Neuroimmunology Branch, NINCDS, NIH
Immunology Section, OSD, NIAID, NIH

LAB/BRANCH

Developmental & Metabolic Neurology Branch, NINCDS

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 5.1	PROFESSIONAL: 4.1	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major protein in myelin of the peripheral nervous system is a glycoprotein. Myelin purified from the central nervous system also contains a glycoprotein, but it is quantitatively a minor component of the isolated myelin. This CNS glycoprotein appears to be selectively concentrated in membranes which are transitional between compact myelin and the oligodendroglial surface membrane. We are purifying this glycoprotein which is in the myelin-oligodendroglial complex so that its chemistry and immunogenicity can be studied. This glycoprotein undergoes a chemical change in developing rat brain which may involve the polypeptide chain or carbohydrate moieties. Since glycoproteins are known to be involved in recognition and contact phenomena, we are investigating its role in developing brain as the oligodendroglial surface membrane makes contact with axons and is spiraled and compacted to form mature myelin. Glycoproteins are also known to be cell surface antigens and receptors for viruses. Therefore, we are investigating the possible role of the myelin-associated glycoprotein in the autoimmune or infectious aspects of multiple sclerosis and other demyelinating diseases.

Project Description:

Objectives: To investigate the biochemistry of cells of the nervous system with particular regard to glycoprotein components and their roles in myelination and demyelination. Other myelin and oligodendroglial proteins and lipids will also be examined with the ultimate objective of understanding the molecular mechanisms of myelin formation and breakdown. Emphasis will be placed on the major myelin associated glycoprotein of the CNS and its role in demyelinating diseases such as multiple sclerosis.

Methods: Specific radioactive sugar precursors are used to label CNS and PNS glycoproteins. Myelin and other subcellular fractions are purified by differential centrifugation on sucrose gradients. Purified myelin is subfractioned into light, intermediate, and heavy fractions with different biochemical and morphological properties. Density gradient centrifugation is also used to isolate other oligodendroglial derived membranes (ODM). Enzyme markers are used to characterize the different subcellular fractions. The membrane-bound proteins and glycoproteins are fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Double label counting techniques are used for detecting the labelled glycoproteins on gels and revealing small differences between samples. Densitometric scanning of gels stained with Fast Green for proteins or periodic acid-Schiff reagent for glycoproteins is used for quantitation of individual protein components. Glycoproteins on gels are also detected by binding of radioactive lectins. Quantitation of individual lipids is carried out by thin-layer chromatographic separation and densitometric scanning of the TLC plates. Purification of the major myelin-associated glycoprotein involves solvent fractionation, preparative polyacrylamide gel electrophoresis, and column chromatographic techniques. Molecular fragments of the isolated glycoprotein are prepared by mild proteolytic or chemical cleavage. Gas liquid chromatography and colorimetric procedures are used for quantitation of individual sugars in glycoproteins. Animals are immunized with the glycoprotein and antibodies are detected by a radioimmunoassay utilizing a $[^3H]$fucose-labeled test antigen and anti-IgG serum.

Major Findings: The principal emphasis has continued to be on the isolation and characterizationof the major myelin-associated glycoprotein. The previously reported procedure of affinity chromatography on Con A-Sepharose followed by preparative gel electrophoresis in the presence of SDS yields a relatively pure preparation of the glycoprotein. However, this procedure is very time consuming and gives a low yield of glycoprotein. A major breakthrough was achieved with the development of a rapid and facile procedure for obtaining a relatively pure preparation of the glycoprotein in good yield. The technique involves solubilization with lithium diiodosalicylate (LIS) followed by partitioning in an aqueous-phenol system. The preparations obtained in this way contain trace amounts of two other glycoproteins and a small amount of lipid which may be specifically associated with the glycoprotein. However, the glycoprotein obtained by this very easy technique appears to be of sufficient purity for many of our chemical and immunological studies.

A simple radioimmunoassay was developed for detecting antibodies to the glycoprotein using an [^{3}H]fucose-labeled test antigen and anti-IgG serum to precipitate antigen-antibody complexes. This assay showed that a rabbit which had been injected with partially purified rat glycoprotein obtained from preparative-SDS gels produced antibodies to the glycoprotein. Another rabbit which had been injected with whole myelin also produced antibodies to the glycoprotein. In contrast, the glycoprotein isolated from rat brain did not produce antibodies in Lewis rats or Strain 13 guinea pigs. These rats and guinea pigs were examined clinically and histologically for signs of experimental allergic encephalomyelitis (EAE) but none were detected. This is not surprising since the rat glycoprotein did not appear to be antigenic in these species.

Experiments with the solubilized glycoprotein showed that it bound tightly to Con A-Sepharose and a variety of other immobilized lectins, including those specific for galactose, fucose, and N-acetylglucosamine. These lectins also agglutinate fragments of isolated myelin. These findings suggest that the glycoprotein is a principal receptor for lectins in myelin and oligodendroglial membranes. In addition, a procedure was developed for binding [^{3}H]Con A to glycoproteins after they have been separated on SDS gels. This technique provides a very sensitive technique for detecting the myelin-associated glycoprotein on gels in cases where material is limited or when in vivo labeling techniques cannot be used.

Studies on the developmental decrease in the apparent mol. wt. of the rat glycoprotein have continued. Regional studies showed that the transition from the larger immature glycoprotein to the smaller mature form correlated well with the caudal-cephalo progression of myelination, adding further support to the concept that the change is important in the mechanism of myelinogenesis. There are now several indications that this developmental change in the glycoprotein involves the polypeptide portion of of the molecule. Thus, very little difference in the sizes of the glycopeptides could be detected by gel filtration after exhaustive digestion of the polypeptide with pronase. Also the mature and immature glycoproteins could not be separated by affinity chromatography on a variety of immobilized lectins. Proteolytic cleavage and peptide mapping experiments are underway in an attempt to elucidate the nature of the difference in the polypeptide chain. Trypsin treatment of myelin fragments converts the 100,000 dalton mature glycoprotein to a molecule of about 80,000 daltons. Treatment of the glycoprotein isolated by the LIS-phenol procedure with a variety of proteases yields 5 to 7 peptide fragments. The peptides produced from the mature and immature forms of the glycoprotein are currently being compared.

We have completed our biochemical characterization of myelin-related membrane fractions from normal rats. A fraction referred to as "$W_1$3" is isolated by density gradient centrifugation from the material released when myelin is osmotically shocked with water. This fraction contains levels of the glycoprotein, the major Wolfgram-protein and 2'3'-cyclic nucleotide 3'-phosphohydrolase which are about 3-fold higher than those of myelin. It contains relatively little basic protein and proteolipid and consists primarily of single membranes and vesicles. It is probably enriched in membranes which are derived from the oligodendroglial plasma membrane but are not compacted into multilamellar myelin, including the processes, the inner and outer surfaces of the myelin sheath, and the paranodal membranes. Another fraction of myelin-related membranes which floats on 0.32 M sucrose was previously studied in hexachlorophene intoxicated rats. We have now shown that small amounts of a similar fraction (F) can be isolated from normal brain. It is particularly deficient in proteolipid protein and the glycoprotein, but contains substantial amounts of other myelin components. It seems likely that the "F" fraction is a form of degrading myelin. Similar $W_1$3 and F fractions can be isolated from bovine and guinea pig brain.

We have now finished our biochemical comparison of mature Xenopus and human CNS myelin. There are quantitative differences in the lipids but both contain cholesterol, cerebroside, sulfatide, and phospholipids. Like human myelin, the Xenopus myelin contains proteolipid and basic protein, but the basic protein is of slightly larger mol. wt. There are differences in the minor high mol. wt. proteins and glycoproteins in the two species. The glycoproteins in Xenopus are considerably more heterogeneous than those in mammalian myelin, although there is a component which coelectrophoreses with the principal mammalian glycoprotein.

In addition, the major protein in Xenopus sciatic nerve myelin is a glycoprotein as is the one in mammalian peripheral myelin. The biochemical similarities between Xenopus and human CNS myelin support the appropriateness of Xenopus optic nerve as a test system for demyelinating agents which is being used by Dr. Webster and colleagues in LNNS.

Significance: The probable localization of the major myelin-associated glycoprotein of the CNS in oligodendroglial derived surface membranes, as suggested by the subfractionation and surface probe studies, has important implications for processes of myelination and demyelination. In this localization the glycoprotein would be accessible for interactions with other cells and with pathological agents such as antibodies and viruses. With regard to myelin formation, there is considerable evidence in the literature indicating that cell surface glycoproteins are involved in recognition phenomena and in specific interactions between cells. The myelin-associated glycoproteins of the CNS could be involved in interactions between oligodendroglial and axonal membranes or between different layers of myelin as they are spiraled and compacted. Many demyelinating diseases such as multiple sclerosis are believed to involve autoimmune and/or viral processes. Membrane glycoproteins are known to be cell surface antigens and receptors for viruses. Therefore, it is possible to suppose that the myelin-associated glycoprotein could be directly involved in demyelinating diseases. Our demonstration that this glycoprotein is antigenic enhances this possibility. For example, in multiple sclerosis a viral induced change in the sugars on the glycoprotein could cause it to be recognized as a foreign antigen and subject to autoimmune attack. For these reasons, information about the chemistry immunogenicity, and localization of myelin-associated glycoproteins will increase our understanding of the molecular mechanisms of myelinogenesis and demyelination. Development of the convenient LIS-phenol procedure for its isolation is a major step toward obtaining more information of this type. Application of the procedures for isolating oligodenglia-derived membranes to pathological tissues should also enhance our understanding of the biochemical parameters of demyelinating conditions.

Proposed Course: One or more additonal purification steps will be developed to remove the minor contaminants from the relatively pure glycoprotein preparation which we can now obtain readily by the LIS-phenol procedure. We will also isolate and characterize fragments of the glycoprotein prepared by proteolytic or chemical cleavage. These smaller molecules may be easier to work with than the intact glycoprotein. In these studies, emphasis will be placed on elucidating the chemical cause for the developmental change in molecular weight. Carbohydrate and amino acid analyses will be done on the intact glycoprotein and fragments with the ultimate objective of determining its overall molecular structure.

Antisera to the glycoprotein isolated from different species by the LIS-phenol procedure will be prepared. We will also determine if animals with EAE induced by whole CNS tissue have antibodies to the glycoprotein. The sera will be tested for demyelinating activity on cultured brain explants in collaboration with Dr. Frederick Seil of Stanford University. These experiments are designed to determine if the demyelinating factor in the serum of multiple sclerosis patients or animals with experimental demyelinating diseases such as EAE is an antibody to the glycoprotein. Finally, multiple sclerosis patients will be tested for humoral or cellular immunity to the glycoprotein.

Antisera to the glycoprotein will be used in immunohistochemical techniques to more precisely localize it in myelin and oligodendroglial membranes. We will continue to study the subfractions enriched in oligodendroglial-derived membranes. The fractions from normal brains will be examined by specialized morphological techniques such as scanning electron microscopy and freeze fracture in order to better correlate structural and chemical parameters. The subfractionation studies will also be extended to demyelinating CNS tissue to obtain information about biochemical changes associated with myelin breakdown . Particular emphasis will be placed on chronic EAE which may be a better model for multiple sclerosis than the usual acute form.

Publications:

1. Everly, J. L., Quarles, R. H., and Brady, R. O.: Proteins and glycoproteins in myelin purified from the developing bovine and human central nervous systems. J. Neurochem. 28: 95-101, 1977.

2. Poduslo, J. F., Everly, J. L., and Quarles, R. H.: A low molecular weight glycoprotein associated with isolated myelin: distinction from myelin proteolipid protein. J. Neurochem. 28: 977-986, 1977.

3. Quarles, R. H.: The biochemical and morphological heterogeneity of myelin and myelin-related membranes. In: Kumar, S. (Ed.): Biochemistry of Brain. Oxford, Pergamon Press, in press.

4. Quarles, R. H. and Everly, J. L.: Glycopeptide fractions prepared from purified central and peripheral rat myelin. Biochim. Biophys. Acta 466: 176-186, 1977.

5. Quarles, R. H. and Pasnak, C. F.: A rapid procedure for isolating the major glycoprotein from purified rat brain myelin. Biochem. J. 163: 635-637, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02024-05 DMN

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cerebral Strokes in Patients with Primary Hyperlipoproteinemia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Anatole S. Dekaban, M.D., Ph.D.	Associate Chief,	DMN	NINCDS
Other:	Jan K. Steusing	Research Assistant	DMN	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.2	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Therapy of severe strokes is generally not very satisfactory and therefore prevention of the cerebrovascular disease is very important. This investigation consists of two phases: (A) establishment of relative frequency in different types of familial hyperlipoproteinemia, (B) institution of appropriate dietary regimen and pharmocological agents aimed at reducing the excessive levels of cholesterol and/or triglycerides in various types of hyperlipoproteinemia as a preventive measure. The first phase of this project is currently under intensive study using clinical, genetic and biochemical methods.

Project Description:

Objectives: Of various factors predisposing to atherosclerosis one of the most important is persistent elevation of plasma lipids whether exogenous - dietary, or endogenous - hereditary hyperlipidemia. It is our intention to study the content and composition of partitioned lipids and lipoprotein patterns in patients with transient or definitive strokes in order to identify the subjects with familial hyperlipidemia. These patients will be studied biochemically in depth and the type and relative incidence of various hyperlipidemias established.

Material: Large number of patients with transient cerebral ischemic attacks or definitive strokes are examined in ambulatory setting and blood drawn following 12-14 hours fasting for determination of partitional lipids and lipoproteins.

Methods Employed:

1. Present and past medical history and genetic history.
2. General medical and neurological examinations.
3. Special examination of the retinal blood vessels and peripheral arteries.
4. Radiological, electroencephalographic, electrocardiographic, and usual routine laboratory surveys of blood and urine.
5. When medically indicated, cerebral angiography and brain scan are performed.
6. Determination of plasma partitioned lipids using established in our laboratory biochemical procedures.
7. Determination of plasma lipoproteins by paper electrophresis, and when indicated by polyacrylamide gel electrophoresis and density gradient centrifugation.

Major Findings: Determination of lipoprotein patterns and of major plasma lipids was carried out in 171 patients with strokes. Of these, 22 had hyperlipoproteinemia (HLIPR) by paper electrophoresis and by elevation of principal plasma lipids (either cholesterol over 300 mg/100 ml or triglycerides over 200 mg/100 ml or both these components). More than two-thirds of the patients had at least one close relative with elevated blood lipids. Using criteria of the World Health Organization, these patients were classified as follows: 5 had HLIPR type IIa, 8 had HLIPR type IIb, 3 had HLIPR type III and 6 had HLIPR type IV. Phospholipids showed relatively little change from the values of normal controls. The numerical distribution of patients with stroke and HLIPR into the four different types corresponds quite well with the approximate frequency of these types of HLIPR in the general population. Thus, this study does not indicate that the patients with a particular type of HLIPR are at a greater risk to have a stroke than those belonging to other types. Therefore in utilizing dietary regimen and appropriate drugs as preventive measures the patients of all types of hyperlipoproteinemia should be enrolled in the study.

Project No. Z01 NS 02024-05 DMN

Significance to Bio-Medical Research and the Program of the Institute

Our Institute has a clearly defined obligation to investigate the etiology, pathogenesis and preventive measures of cerebrovascular disease. Primary hyperlipoproteinemia is associated with permanent elevation of principal plasma lipids: cholesterol or triglycerides or both. These patients are under high risk for development of strokes. For this reason young patients with familial hyperlipoproteinemia are particularly suited for studies on cerebrovascular disease with a view of prevention.

Proposed Course of the Project: The objectives of the first part of the project have been accomplished and we will have to make decision now on how to organize the second phase: preventive management of subject with familial hyperlipoproteinemia.

Publications:

1. Dekaban, A.S., Stevens, H., and Steusing, J.K.: Hyperlipoproteinemia and Cerebral Strokes. Can. J. Neurological Sci., 4: 13-17, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02129-03 DMN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical Characterization of Mucopolysaccharidosis Type VI (Maroteaux-Lamy).

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: George Constantopoulos, Ph.D.	Biochemist	DMNB	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH Developmental and Metabolic Neurology Branch

SECTION Clinical Investigations Service

INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated and no work has been done on it.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02162-03 DMN

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Synthesis of Compounds Analogous to Glycolipids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Gal, Section Head, Research Chemist,	DMN, NINCDS
OTHER:	F. Fash, Bio. Lab. Technician,	DMN, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Neurochemical Methodology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.6	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Compounds were synthesized which are glycolipid analogs and which give a chromophoric moiety upon enzymatic hydrolysis. These compounds are used in enzymatic research and as diagnostic reagents in Niemann-Pick, Gaucher's and Krabbe's disease.

Project Description:

Objectives: The compounds to be synthesized in the framework of this project are molecules similar to glycolipids which when cleaved enzymatically provide a chromophore useful for the diagnosis of lipid storage diseases and for the identification of heterozygous carriers.

Methods and Major Findings: Further work was done related to improvements of the synthesis of 2-hexadecanoylamino-4-nitrophenyl phosphorylcholine (HNP). This substance resembling sphingomyelin but having a benzene ring instead of the aliphatic chain gives due to its nitrophenyl moiety intense coloration upon enzymatic attack. It is a reliable chromogenic substrate used for assaying sphingomyelinase activity in diverse human tissue samples. It is used for the diagnosis of homozygotes and detection of heterozygous carriers of Niemann-Pick disease. This compound was synthesized by Calbiochem and is sold now by that company. We also developed a simplified synthesis of HNP based on free phosphorylcholine as the starting material. This improvement could be realized due to the availability of free phosphorycholine for which compound we developed a practical synthesis. Based on the chemistry of HNP, the research on non-radioactive substrates were extended to other lipidoses. Compounds were synthesized which could be used as substrates for measuring gluco and galactocerebroside levels in tissue extracts. 2-Hexadecanoylamino-4-nitrophenyl glucoside showed to be a useful compound for the diagnosis of Gaucher's disease. 2-Hexadecanoylamino-4-nitrophenyl galactoside can be used for the diagnosis of Krabbe's disease. Work is also going on on the synthesis of a substrate which would make feasible the diagnosis with a chromogenic substrate in Farber's disease, a disease characterized by the lack of ceramidase.

Significance: These new compounds were thoroughly tested and they have been found useful for the diagnosis of lipid storage diseases. These findings are a major breakthrough because the radiolabeled products are scarce, expensive, and not widely available. The chromogenic substances can be used and easily handled by practitioners and clinical chemists with no danger of radioactive contamination and they eliminate the necessity of costly and complex radioactive scanning techniques.

Proposed Course: Based on the basic idea established by this project, compounds will be synthesized with chromophoric moieties which hopefully will be specific for the detection of other enzyme deficiency disorders.

Publications:

1. Brady, R. O., Pentchev, P. G., Gal, A. E., Quirk, J. M., Hibbert, S.R., Mook, G. E., Kusiak, J. W., Tallman, J. F., and Dekaben, A. S.: Enzyme replacement therapy for sphingolipidoses. In Volk, B. W. and Schneck, L. (Eds.): Current Trends in Sphingolipidoses and Allied Disorders. New York, Plenum Press, 1976, pp. 523-532.

2. Gal, A. E., Pentchev, P. G. and Fash, F. J.: A novel chromogenic substrate for assaying glucocerebrosidase activity. Proc. Soc. Exp. Biol. Med. 153: 363-366, 1976.

3. Gal, A. E. and Fash, F. J.: Preparation of phosphorylcholine from phosphorylcholine chloride calcium salt. Lipids 12: 314-315, 1977.

4. Gal, A. E., Brady, R. O., Pentchev, P. G., Furbish, S. F., Suzuki, K., Tamaka, H., and Schneider, E. L.: A practical chromogenic procedure for the diagnosis of Krabbe's disease. Clin. Chim. Acta 77: 55-59, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02163-03 DMN

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of Special Analytical Methods and Preparative Techniques to Investigate the Etiology and Therapy of the Sphingolipidoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Gal, Section Head, Research Chemist	DMN, NINCDS
OTHER:	F. J. Fash, Biol. Lab. Technician	DMN, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Neurochemical Methodology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. Enzyme purification was studied by affinity chromatography. The lipid content in human tissues, the diagnosis of lipid storage diseases by gas, thin-layer chromatography and other techniques were studied at the microgram level. Also preparative work was done and used in connection with further synthetic work and for the preparation of lipid standards.

Project No. Z01 NS 02163-03 DMN

Project Description:

Objectives: To develop techniques by which the separation and chemical analysis of biologic materials related to sphingolipidoses can be advanced. This work involves the following approaches: 1. Improvement of techniques leading to the separation of enzymes. 2. Development of ultramicro analytical methods for the determination of lipids in biological materials.

Methods: 1. Work on the separation of enzymes is related to affinity chromatography, a technique whereby an enzyme is temporarily fixed to a column containing a molecule (ligand) which reacts with an enzyme by immobilizing it. An insoluble inert support (such as agarose) is bound to a spacer arm which contains the ligand. The latter is usually a synthetic compound similar in its complexity to the substrate with which the enzyme interacts. A great number of ligands were synthesized by us and the corresponding affinity columns were assembled. 2. The development of methods for the determination of lipids in small samples of biological materials of human origin such as erythrocytes, leukocytes, fibroblast serum, cerebrospinal fluid, urine or biopsy samples from kidney, liver and brain. The individual sphingolipids are present usually only in submicrogram quantities in these samples. For the separation of such lipids, thin layer and gas chromatographic procedures combined with column-liquid chromatography was used.

Quantitive evaluation was made by scanning of the thin-layer plates or by gas chromatography. Much work was done in areas not covered by existing literature references.

Major Findings: Improved purification of the enzymes was achieved by using affinity chromatography systems. Considerably more work has to be done before the advantage of these procedures could be evaluated in gains in man-hour work. Gas chromatography of glucose originating from lipids could not be determined previously. This problem was solved by us. Also a new thin-layer chromatography system was developed which resulted in more reliable results using only small amounts of specimen. A novel technique was developed in which lipids present in the same sample (but not attacked by the exogenous enzyme) were used as internal standards. Improved analytical techniques showed practical results particularly in the studies related to replacement therapy of enzymes where the decrease of lipid levels in the liver and erythrocytes of patients could be established and through these procedures, evaluation of the therapeutic administration of enzymes can be assessed.

Significance: The purification of the missing enzymes required for the therapy of the lipid storage diseases is a complex, tedious, and costly procedure. The use of affinity chromatography should provide a significantly simplified method. The identification of accumulated lipids in human tissues for the diagnosis and control of inherited lipid diseases is dependent on the sensitivity of the analytical techniques. The importance of accuracy in working with trace amounts of material in biological specimens necessitates improved techniques at the submicrogram level.

Proposed Course: Efforts to improve the purification of enzymes by affinity chromatography or by other chemical operations will continue as well as by utilizing other advanced techniques. Much more work has to be done in relation to the improvement of microanalytical procedures; for example, the ultramicrodetermination of aminosugars and sialic acid needs further development. Some of the existing methods are too complex and their simplification will be investigated. The application of other techniques including high speed (or pressure) liquid chromatography or the use of mass spectroscopy will be explored.

Publications:

See Project No. Z01 NS 00815-17 DMN.

ANNUAL REPORT

July 1, 1976 to September 30, 1977
Laboratory of Neuropathology and Neuroanatomical Sciences, IRP
National institute of Neurological and Communicative
Disorders and Stroke

Igor Klatzo, Chief

As in previous years, the main research efforts of this Laboratory were concentrated toward further elucidation of pathophysiology of cerebral ischemia, cellular mechanisms of demyelinating diseases, structural basis of synaptic transmission, and aspects of normal and pathological passage of various substances within the nervous system.

The Section on Neurocytobiology has continued to investigate the transport phenomena, as well as the enzymatic and metabolic changes in cerebral ischemia, using the carotid occlusion in the Mongolian gerbil as the main experimental model. It was demonstrated that ischemia has selective and diverse effects on the behavior of the blood-brain barrier (BBB). Thus changes in water and electrolyte distribution preceded increased permeability to small molecular weight substances and it was followed later by a transient opening of the BBB to proteins. The latter event was associated with increased diffusion of 2 deoxy-D-glucose (^{3}H 2-DG) into the affected tissue, whereas an increase in the facilitated, carrier mediated transport of glucose corresponded to the period of abnormal passage of smaller molecules.

The post-ischemic increase in the transport of ^{3}H 2-DG was further supported in studies in vitro on isolated cerebral capillaries which revealed an increased ^{3}H 2-DG uptake in capillaries obtained at various periods following release of the carotid occlusion. These and the other studies on the effect of hypoxia suggested strongly that the facilitated transport of glucose and glucose analogues is an oxygen dependent phenomenon. Furthermore other experimental data indicated that the capillary transport of ^{3}H 2-DG may be related to fatty acid metabolism. The studies on several enzymes assayed comparatively in cerebral capillaries and brain parenchyma revealed that the former are affected earlier than the latter in cerebral ischemia.

Otherwise, enzymatic assays showed that monoamino-oxidase (MAO) activity could not be responsible for the observed changes in biogenic amines in cerebral ischemia. Ischemia also has no effect on enzymes involved in the metabolism of the cholinergic neurotransmitters. The regional ischemic and post-ischemic cerebral glucose consumption (RCGC), evaluated radioautographically using ^{14}C 2-deoxy-D-glucose was correlated with the histochemical activity of hexokinase and changes of the BBB with regard to Evans blue tracer. The areas of increased RCGC appeared to reflect an altered glucose metabolism with the ischemic tissue and otherwise they were difficult to correlate with hexokinase or BBB changes.

A preliminary success has been recorded in establishing of cerebral endothelial cell cultures which could be used for investigation of the biochemical and physiological properties of cerebral endothelial cells without influence of other cells. A few pure cerebral capillary cultures have been growing slowly as sheets of elongated cells. Other studies in tissue culture demonstrated differences in uptake of various amino-acids between pia arachnoid and skin fibroblastic cultures.

The Section on Cerebrovascular Pathology has been pursuing a variety of approaches toward elucidation of pathophysiological mechanisms involved in the disturbed cerebrovascular permeability and their role and participation in the development of injury to brain parenchyma occurring in a variety of pathological conditions. Correlations of water, electrolytes, BBB changes as well as regional cerebral blood flow (CBF) and regional glucose consumption (the last ones assessed radioautographically) revealed some complex interrelationships between various parameters of experimentation. Differential and selective vulnerability of the BBB was demonstrated by application of different indicators. The regional changes in glucose utilization showed no relationship to the BBB changes and suggested some neurogenic control for metabolic alterations.

An attempt to influence the clinical course and survival following an ischemic insult produced findings which are striking in their statistical significance but remain elusive with regard to elucidation of patho-physiological mechanisms involved. Mongolian gerbils subjected to 15 minute bilateral occlusion of the common carotid arteries showed a 20% survival rate after 30 days. In those animals which had an additional injection of 2 ml of 5% glucose, the survival was even more reduced (appr. 15%). However, gerbils with the same bilateral carotid occlusion and administration of glucose but additionally receiving 0.02 ml Ringer solution injection in the cisterna magna showed a striking survival of over 80%. The reason for such a dramatic difference in mortality following ischemic insult is being presently investigated by assaying various biochemical parameters in different groups of gerbils.

In the Section on Cellular Neuropathology, research on cellular mechanisms of demyelinating diseases has continued. The myelinotoxicity of 88 samples of CSF has been tested with a method developed in this laboratory. Two-thirds of the 33 CSFs that were obtained from patients with a clinical diagnosis of definite multiple sclerosis (MS) had myelinotoxic activity which correlated best with the patient's clinical state at the time of lumbar puncture. If done less then 3 wks after the onset of an acute attack or progressive symptoms, and if the patient had recieved no steroid therapy, more than 90% of the CSFs were positive. Only 33% were myelinotoxic if obtained from patients with definite MS on steroid therapy or from those who were stable clinically or who had progressive disease for more than 3 wks. Significant ($P < .05$) myelinotoxic activity was found in 50% of CSFs from patients with possible MS, 30% of CSFs from patients with optic neuritis and 22% of CSFs from patients hospitalized for other neurological diseases. The purpose of present and future research is to characterize the myelinotoxic factor(s) in CSF from patients with acute attacks of MS and to gain further experience with the test and its specificity.

In the Section on Functional Neuroanatomy, a major research effort is concerned with the structural basis of synaptic transmission. This project seeks to clarify the exact location and mechanism of synaptic transmission in the central and peripheral nervous system. It is carried out by examining synapses with the electron microscope to determine how their structure changes during activity.

Recently, this section published a method for capturing the fleeting structural details in functioning synapses by freezing them very rapidly, thereby avoiding the use of chemical fixatives and cryoprotective agents which alter profoundly the structure of synapses. By freeze-fracturing rapid-frozen neuromuscular synapses, it has been possible to see, and count, synaptic vesicles fusing with the synaptic plasmalemma of synaptic terminals at several different levels of transmitter secretion. It turns out that each quantal secretory event results from the fusion of one synaptic vesicle with the plasmalemma. Since the temporal resolution of rapid freezing in the machine used by this section is less than 2 msec, as measured by a capacitance method developed here this year, the section could hope to follow the fate of synaptic vesicle membrane after vesicles fused with the synaptic plasmalemma. In less than 0.1 sec, the vesicle membrane is completely flattened out into the plasmalemma. Components of the vesicle membrane, appearing as particles after freeze-fracturing, then spread out randomly, finally to be collected a second later in little particle islands which are parts of the coated vesicle system. The final fate of these components of the vesicle membrane is to be reincorporated into synaptic vesicles. This finding of particle recycling extends earlier work of the section showing that local recycling of synaptic vesicles replaces those lost during synaptic activity.

This year a study was completed which applied the freeze-fracture technique to neuromuscular synapses to see why such medically important biological substances as botulinum toxin and black widow spider venom have such profound effects on synapses. Results indicate that the toxin specifically blocks exocytosis, probably by preventing calcium ions from entering the nerve terminal, and that the venom lets calcium ions in to excite exocytosis. One of the most interesting findings, which supports these conclusions, is that the venom can overcome the effect of the toxin, so that the normal distribution of exocytosis reappears. The clinical usefulness of this finding is limited however, unless a way can be found to return the exocytosis to control by nerve impulses arriving at the synapse.

The freeze-fracture technique has also yielded new information about the structure of the postsynaptic membrane. Particulate structures, thought to be receptor molecules within the postsynaptic membrane, appear to be different at each chemical type of synapse. In fact, the new evidence collected this year on ganglionic synapses in squid, insect neuromuscular junctions, and synapses in the mammalian inner ear strengthen our previous hypothesis that these particle aggregates are the sites of receptor-ion channel complexes in the postsynaptic membrane and that their structure is specific for each functionally different type of chemical synapse. While more chemical types of synapses need to be examined, these studies indicate that identification of pharmacological types of synapses in the central nervous might be approached with anatomical techniques.

Several other current studies with the freeze-fracture technique have begun to yield new data on changes in the structure of synaptic membranes during development. At the developing presynaptic membrane, studied in the chick retina, components of the presynaptic specialization appear first in small islands which subsequently fuse to form the large adult specialization. In developing chick muscle in culture, particle aggregates of the type associated with receptors in the adult appear at spots of acetylcholine sensitivity (inferred from binding of fluorescent bungarotoxin) prior to the arrival of the nerve terminals. The changes in synaptic membranes which accompany initial contact between nerve terminals and target cells were studied in cultures of sympathetic ganglia, using a technique for staining cell coats in thin sections. This revealed a new form of junction which formed and disappeared as synaptic junctions formed, suggesting that these initial contact junctions could have a role in the induction of synapse formation. However, careful study with the freeze-fracture technique of similar junctions at sites of future synapses in the developing cerebellum failed to reveal any special structures inside membranes at these initial contact junctions. Thus, it is possible that these structures are confined to the surface coat of developing synaptic membranes.

A high resolution scanning electronmicroscope was acquired in the last year and this instrument was used to reveal the structural organization of surfaces of the postsynaptic membrane at greater levels of detail than has previously been possible. However, this technique is still too new to be certain yet of the significance of the new surface features of the postsynaptic or other membranes which it reveals.

One of the most immediately practical aspects of the current studies of synapses is that they define the normal structure of various types of synapses in a variety of functional states. This knowledge will permit distinction between normal and pathological, as well as between resting and active synapses, with the electron microscope. In structural studies of epileptic brains, it should now be possible to distinguish normally active from pathologically active synapses. Similarly, in diseases involving peripheral nerve-muscle synapses at neuromuscular junctions, it will be possible to distinguish pathological states from changes resulting from increased or decreased activity. The finding that different chemical types of synapses are distinguished by the freeze-fracture technique may contribute to the task of determining the chemical organization of synapses in the central nervous system. Knowledge about the locations and pharmacological types of various central nervous system synapses will make it possible to understand the action of drugs on the brain on a cellular level. A clear understanding of the changes in the pre- and postsynaptic membranes during development may lead to an understanding of the factors inducing synapse formation, and thus the development of and regeneration of synaptic connections in the central nervous system.

A second major project in the Section on Functional Neuroanatomy uses the electron microscope to determine the cellular basis of the blood-brain barrier (BBB) to proteins. Previous studies showed the location of the BBB within blood vessel walls in the parenchyma of the brain. Tight junctions between endothelial cells were shown to make an important contribution to this barrier. More recent studies established the location of these barriers in the organ of Corti in the ear. The structure of the blood vessel wall has also been reexamined in an effort to define the role of the astrocyte sheaths which always surround the endothelium.

Barriers in the organ of Corti of the mammalian inner ear were the object of a collaborative study with the Laboratory of Neuro-otolaryngology. We found that the perilymph is separated from the endolymph by tight junctions between the apices of hair and supporting cells. The freeze-fracture technique showed that these junctions are more extensive and, presumably, "tighter" than in any other tissue studied so far and that they block the passage of proteins from the perilymph to the endolymph. Their unique form also suggests that they might have functions other than to prevent mixing of endolymph and perilymph and, perhaps, are involved in the first steps in the sensing of mechanical displacements in the organ of Corti.

The astrocytes which surround cerebral blood vessels were also reexamined. Previous studies showed that the membranes of perivascular astrocytes have unique assemblies of particles, especially where they face blood vessels. Studies of slices of cerebellum rapidly frozen after incubation in O_2 or N_2 containing artificial cerebral spinal fluid showed that the assemblies are maintained in medium containing O_2 but disappear in the absence of oxygen. This finding suggests that they are a metabolically maintained component of the astrocyte membrane and therefore, possibly the location of pumps which might adjust the intracellular environment of the brain.

These data on the cellular organization of the BBB as well as the anatomical techniques used in these studies, afford a basis to examine a variety of clinical disorders which depend on or produce disruption of the BBB. They also give rise to new ideas about the roles of the various glial cells associated with neurons and about the function of barrier systems in special regions such as the organ of Corti.

A third major interest has been the formation of neurotrophic slow viruses, a project undertaken in collaboration with the Infectious Diseases Branch. It was hoped to elucidate how neurotrophic viruses form in infected cells but also expected that infected cells would serve as good experimental subjects with which to develop methods to see how components of their membrane, such as viral antigens or pharmacological receptors, are assembled in special regions of the neuronal surface. Much of this work was reported in final form this year and we completed a search for signs of slow virus infection in brains of mice infected with scrapie. The scrapie lesions were identified for the first time in freeze-fractured brains, but no details of membrane structure that could be attributed directly to the scrapie agent were found. Therefore, the scrapie agent is either very dilute, even within the scrapie lesions, or it does not penetrate to the hydrophobic interiors of membranes where it would be revealed by the freeze-fracture technique.

In the Section on Neurocytology, the regeneration of superior cervical ganglion (SCG) fragments, transplanted to cerebrospinal fluid (CSF) compartments, is being followed in an attempt to direct, eventually, their regenerating axons to specific targets. This project, begun 5 months ago, has already shown that the donor rat must be about 21 days old before its SCG will "take" and grow in its own or another rat's CSF space. The SCG is placed on the floor of the IV ventricle and over the pia of the medulla, care being taken not to injure either ependyma or marginal glia in young (6 to 14 day old) rats. The ganglion cells not only survive the longest period, 90 days, so far examined but, surprisingly, the SCG becomes incorporated within the stroma of the choroid plexus. In some way, the choroidal epithelium must loosen its tight junctions to enclose the SCG fragment which becomes "capped" by both choroidal epithelium and ependyma. The SCG stroma also becomes confluent with the parenchyma of the medulla at points where the ependymal cells appear to have become disorganized, perhaps separated. The regenerating SCG implants are well vascularized. To be tested is the supposition that the vessels are fenestrated and permeable to peroxidase. If they are permeable, then blood-borne protein such as nerve growth factor should be able to reach the ganglion cells.

The orthograde, intra-axonic transport of protein has been compared with the retrograde route in the hypoglossal nucleus and the neurosecretory system. Horseradish peroxidase (HRP) in low volume (10 to 30 μl) and high concentration (30%) is infused ventrico-cisternally in mice. From 5 minutes to 48 hours later, the HRP is pinocytosed directly by dendrites and somata of neurons. At a given time, however, the amount of HRP in perikaryal lysosomes in never as great as after retrograde transfer from the blood. However, the orthograde transport of HRP in neurosecretory axons can be enhanced by hyperosmotic stress. Mice, given a 1.5% NaCl solution to drink for 5 days, transport more HRP within hypothalamohypophysial axons than in non-stressed mice. More terminals of these axons in the posterior lobe of the pituitary gland are also labeled. The same organelles - cisterns of the smooth endoplasmic reticulum and vacuoles - are involved in both ortho- and retro-grade intra-axonal transport. In axon terminals, labeled vacuoles are the same size as some neurosecretory granules. However, since hemorrhage, which causes release of neurosecretory material, does not cause the release of HRP from these terminals, the labeled vacuoles must represent a separate group of organelles from neurosecretory droplets.

The freeze-fractured membranes of subpial (marginal) and perivascular astrocytes contain orthogonal arrays or assemblies of small particles as do ependymal cells. In some ependymal cells, especially near points of branching, the assemblies form long trains. Assemblies do not occur in choroid plexus epithelium. The functions of the assemblies are unknown but might have something to do with secretion or absorption of fluid and solutes. Solutions of 10^{-4} M ouabain and of acetazolamide, which reduces CSF production, are being infused ventriculo-cisternally in mice. The section is also attempting to change the number or configuration of the assemblies by causing marginal astrocytes and ependyma to proliferate or hypertrophy after painting 30% to 66% gels of aluminum hydroxide onto the floor of the IV ventricle and the dorsal surface of the medulla.

In order to determine whether such assemblies are present in astrocytes and ependyma cultured in vitro we must first identify the cells as glial or fibroblastic. To make this distinction, a cytoimmunochemical technique is used whereby fixed cells in situ and in vitro, are labeled with an antibody to a "non-neuronal protein" (NNE) which has been found to label astrocytes in fixed, wax-embedded slices of brain. The NNE also stains ependyma and choroid plexus epithelium and is an enolase found in liver. A "neuron-specific protein" (NSP) is also an enolase, not found in liver, which stains many neurons throughout the brain.

Hypertension, induced by the intravenous injection of aramine or the rapid infusion (0.6 ml per second) of a bolus of saline into one carotid artery of rats results in the escape of horseradish peroxidase (HRP) from cerebral blood vessels. Other investigators have recently claimed that the HRP crosses affected cerebral capillaries by vesicular transport. The Neurocytology section has found that the injection of hyperosmotic (2.0 M) urea in rabbits also opens the blood-brain barrier to HRP by opening endothelial tight junctions. However, when hyperosmotic (1.4 M) arabinose is injected into one carotid artery of rats, very few endothelial junctions were penetrated by the HRP. In order to test whether vesicular transport could account for the exudation, a key control experiment has been performed. One minute after the intracarotid infusion of arabinose, the brain is fixed with aldehyde and, one to three hours later, when vesicular transport can no longer occur, HRP was infused into the aorta of the carcass. Exudates appeared in the arabinose treated side of the fixed brain. A number of wide separations, probably at tight junctions, are found in such brains. We conclude, therefore, that HRP crosses endothelium through damaged, i.e., widely separated endothelial cells or by actively formed channels. The channels remain patent long enough to be fixed in the open position. Vesicular transport - the formation of individual vesicles at one face of the cell, their migration and coalescence at the opposite face - cannot account for the exudates.

The permeability of vessels to fatty acid (FA) has been investigated by injecting unphysiologically high but detectable concentrations of linolenate into one carotid artery of rats. Osmiophilic droplets are not only randomly distributed in cells but are found specifically within pinocytotic pits of endothelium, in contact with mitochondria of the smooth muscle coat of arterioles, but almost never within the nucleus of any cell type. In some perivascular autonomic nerve terminals and in some boutons of cerebral parenchyma, synaptic vesicles are stained by the FA. The postsynaptic web is especially electron dense at many other contacts.

The Section on Experimental Neuropathology has been engaged in studies on Production of cerebral lesions mimicking those of human cerebral fat embolism, Mechanism of cerebral hemorrhages, Sites of origin of individual branches of the facial nerve, Methods to improve fixation by perfusion, and Brains from Guam (in collaboration with the Laboratory of Central Nervous System Studies).

Experimental cerebral fat embolism following intravenous or intracardial injection of oil produced lesions which usually affect large parts of the brain. The intraperitoneal injection of oil induced myriads of minute lesions similar to those of human cerebral fat embolism. Unfortunately, this experimental model could not be utilized because oil was only on rare occasions transferred to the systemic circulation.

Since the brains of the affected animals, which were fixed by perfusion, contained myriads of petechial hemorrhages, study of oil embolism material was continued by injecting oil into the systemic circulation, in an attempt to clarify the mechanism of cerebral hemorrhages. Besides pericapillary effusions of erythrocytes, there were hemorrhagic infarctions in the brains of cats sacrificed by perfusion two or more days after injection of oil. By changing the pressure of perfusion, the interval between injection and perfusion, and the animal species, as well as by tagging the erythrocytes with Evans blue shortly before perfusion, one may be in a position to assess some of the causative factors. These results may offer a better basis for understanding the mechanism involved in the formation of similar hemorrhages in man and to estimate their role in so-called "hemorrhagic" diseases and in the terminal events of a malady as the cause of death. Until conclusive results have been obtained, it is premature to formulate any hypothesis about their formation and prevention.

Efforts to improve current methods of perfusion have been aimed at preventing the formation of recently identified "solitary" dark neurons; these cells are recognized as being caused by post-mortem trauma to unfixed normal neurons. After use of rapidly acting fixatives, the necessity of delaying the autopsy several hours, recommended for avoiding the massive formation of dark neurons, has been confirmed. Other modifications are tested for the purpose of optimizing the histochemical glycogen reaction. In spite of use of material fixed by perfusion, the results are inconsistent, which may explain controversial observations on glycogen content in neurons reacting to severance of their axons. An improved histochemical technique is essential for an assessment of the vulnerability of the glycogen-rich neurons to severance of their axons as well as for an estimation of the effect of cortisone on glycogen deposition in the brain.

The method of retrograde axonal flow of horseradish peroxidase is currently being adopted for localization of perikarya from which axons to different muscles of the face emanate. According to the scant literature, the opinion about the site of origin is not unanimous. According to personal observations, the extent of acute neuronal chromatolysis after transection of a small branch of the facial nerve is larger (global reaction) than that of neurons tagged by retrograde flow of peroxidase (focal reaction).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01999-05 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transport studies in ischemic cerebral edema.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Fujimoto	Visiting Scientist	Univ. W. Ontario
OTHER:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

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Section on Neurocytobiology

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.55	.2	.35

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The passage of small and large molecular weight substances across the blood-brain barrier (BBB) was investigated during development of biphasic ischemic edema in the brain. The initial increase in cerebral water content (first phase) preceded the increased BBB permeability to Evans blue albumin complex, RISA, dextran and sucrose. The BBB change for large molecular weight substances was transient and most likely responsible for additional brain swelling (second phase). Moreover, the facilitated transport of 2-deoxy-D-glucose was altered and included an increased passive diffusion of the hexose from blood to brain during the second phase of ischemic cerebral edema.

Project Description:

Objectives: Cerebral edema was found to be the most common feature in gerbils subjected to various periods of cerebral ischemia (Ito, U., Spatz, M., Walker, J. T., jr., and Klatzo, I., Acta Neuropath. 32: 209-223, 1975). Moreover, in short-term ischemia, a biphasic change in cerebral water content was observed in the affected hemisphere which appears to be related to the absence or presence of increased blood-brain barrier (BBB) permeability to Evans blue albumin complex after the cerebral circulation was reestablished (Fujimoto et al., in Dynamics of Brain Edema, Berlin Heidelberg, Springer-Verlag, 1976, pp. 171-180). Therefore, this study was undertaken to investigate the passage of small and large radiolabeled molecules with and without Evans blue from blood to brain in order to elucidate the functional status of the BBB and possible mechanism responsible for the development of cerebral ischemic edema.

Methods Employed: Several groups of gerbils were subjected to 1 hour common carotid clipping and various periods of clip release. Only animals with neurological signs were injected with the radiolabeled substances with and without Evans blue via femoral vein 20 minutes prior to the termination of the experimental period. After decapitation, the brains were removed quickly and each hemisphere was divided into two parts. The cerebral samples were weighed and processed for liquid scintillation counting or gamma counting.

Major Findings: The highest incidence and the greatest increase in sucrose, dextran and RISA uptake of the ischemic hemisphere was demonstrated 10 hours after the release of carotid artery occlusion. The increased relative percentage of cerebral dextran uptake corresponded well with the grossly observed extravasation of Evans blue albumin complex in the affected brain. However, the increased sucrose uptake was observed prior to and later than the increased dextran and RISA uptake (5 and 20 min after clip release). The restoration of cerebral blood flow following 1 hour of ischemia not only affected the permeability of BBB to small and large molecules but also involved the carrier mediated process as was evident by increased ^{3}H 2-DG uptake 5 and 20 min after clip release. This increased ^{3}H 2-DG brain uptake could be inhibited to the same degree as in the controls by 60 mM cold 2-DG. However, at 10 hours of cerebral recirculation of the blood the ^{3}H 2-DG couldn't be inhibited, indicating the presence of 2-DG diffusion.

Significance to Biomedical Research and the Program of the Institute: The basic comprehension of the blood-brain barrier functions concerned with the passage of nutrient and non-nutrient substances from blood to brain following cerebral ischemia is of major importance (1) for the understanding of the mechanism responsible for the development of ischemic edema, as well as elucidating other pathophysiological processes occurring in cerebrovascular disease and many other neurological disorders, and (2) for selecting the best therapeutic approach to a given disease.

Proposed Course of the Project: This model will be useful to study the facilitated transport of different substances, which may be altered in ischemic edema.

Publications:

Spatz, M., Fujimoto, T. and Go, G. K.: Transport studies in ischemic cerebral edema. In Pappius, H. M. and Feindel, W. (Eds.): Dynamics of Brain Edema. Berlin Heidelberg, Springer-Verlag, 1976, pp. 181-186.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02000-05 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Brain edema in cerebral ischemia of gerbils

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimentally, cerebral ischemic edema can be produced easily in 30% of Mongolian gerbils by unilateral ligation of the common carotid artery. The cerebral water content was assessed by the determination of wet and dry weight and the specific gravity of the tissues. In short-term ischemia of 1 hour duration, the cerebral water content was increased, but did not show any progression until 10 hours after the release of arterial occlusion. At this time, the percent of water content was more pronounced coinciding with the increased permeability of blood-brain barrier to Evans blue tracer. A week later only half of the animals showed recovery. In long-term ischemia, progressive accumulation of water content was observed with prolonged duration of ischemia.

Project Description:

Objectives: In human cerebral ischemia, brain edema is considered to be an important factor in causing mortality (Shaw, C., Alvord, E., and Berry, R., Arch. Neurol. 1: 161-177, 1959). Experimentally, cerebral ischemia can be easily produced in Mongolian gerbils by ligation of a single common carotid artery (Levine, S., Payan, H., Exp. Neurol. 16: 252-255, 1966; Kahn, K., Arch. Path. 69: 544-553, 1972; Ito et al., Acta Neuropath. 32: 209-223, 1975). In our recent studies of ischemic brain edema, in gerbils, a progressive decrease in percent of dry weight (i.e., an increased water content) with a net loss of potassium and with a net gain of Na was observed in the affected hemisphere as compared to the unaffected and the control hemisphere in long-term ischemia. The present investigation has been a continuation of this study to determine the changes occurring in the brain after short period of ischemia and various recovery periods as compared to the long period of ischemia.

Methods Employed: Several groups of adult gerbils were subjected to unilateral clipping and clip release of the left common carotid artery for various periods of time. Only the gerbils with definite cerebral symptoms were selected for this study. Two different methods were used for the determination of cerebral water content: (1) wet and dry weights, and (2) specific gravity, which allows the assay of small samples of brain tissue and therefore, regional alteration in the water content can be established (Nelson et al., J. Appl. Physiol. 30: 268-271, 1971).

Major Findings: An increase in water content of the brain tissue was observed already after 15 minutes of common carotid artery occlusion. In short-term ischemia of 1 hour duration, the cerebral water content (determined by both methods) was increased, but shows little variations until 10 hours following clip release. At this time, an increased BBB permeability to Evans blue albumin complex was seen in the brain. A week later, the examined animals can be divided into two groups of which one shows complete recovery only. In long-term ischemia, the reduction of the specific gravity in the cortex, basal ganglia and hippocampus progressed with the length of occlusion as was previously observed by the wet and dry weight determination of water content. The contributing factors in the recovery periods such as increased blood-brain barrier permeability and tissue necrosis, which most probably are responsible for secondary increase in cerebral water content, are under evaluation.

Significance to Biomedical Research and the Program of the Institute: Cerebral edema occurs as one of the major complications of many neurological disorders such as ischemia, trauma, tumors, chemical poisoning, and others. The basic understanding of the type of edema and its development is very crucial for the clinician who is faced not only with the diagnosis, but with the appropriate selection of treatment. Thus, various investigations of this problem are essential for finding the factor or factors responsible for the occurrence of cerebral edema and its treatment.

Proposed Course of the Project: The study of brain edema in ischemia will be concerned with the continuous effort to differentiate the early cytogenic from the secondary vasogenic component of the cerebral edema. The investigation will include electron microscopic and radioisotopic evaluation of the ischemia brain in gerbils.

Publications: See Project No. Z01 NS 01999-05 LNNS

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02001-05 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Amino acids transport in hypoxia, hypercapnia and hypocapnia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
OTHER:	T. Fujimoto	Visiting Scientist	Univ. W. Ontario
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

University of Western Ontario, London, Ontario, Canada

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been discontinued for the present time.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02083-04 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Uptake of glucose analogues in cerebellar culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Renkawek	Assoc. Professor	Inst. Neuropathol., Warsaw
OTHER:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	M. R. Murray	Research Biologist	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
Institute of Neuropathology, Polish Academy of Sciences, Warsaw, Poland

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.15	.6	.55

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The myelinated organotypic cerebellar cultures composed of glia, neurons and granular cells, but almost devoid of capillaries, took up the non-metabolizable 3-0-methyl-D-glucose (3-MG) and the partially metabolizable 2-deoxy-D-glucose (2-DG) by NaCl independent carrier mechanism. The Km that is the apparent concentration of half maximal uptake of ^{3}H 3-MG and ^{3}H 2-DG was 11 μM and 4 μM, respectively.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The changes in brain uptake of nutrients and non-nutrient substances in disease processes may depend on the integrity of the cerebral capillaries and/or neurons and glia. Since organotpyic cerebellar cultures are composed of various cellular elements, we undertook to explore the possibility of utilzing organotypic CNS explants to study the permeability of brain cells to sugars in the living intact state. This study is a continued investigation of several parameters of glucose analogues uptake by cerebellar cultures in order to characterize its uptake under physiologic conditions.

Methods Employed: The Maximow technique was used for cultivation of explants from newborn rat cerebellum. The cultures were fed twice a week with equal parts of Eagle basal media containing glutamine, fetal calf serum, bovine serum ultrafiltrate, Simms' balanced salt solution (BSS) supplemented with 10% dextrose. Fourteen day old explants, washed with dextrose-free BSS, were used for ^{3}H methyl-D-glucose or ^{3}H 2-deoxy-D-glucose (10 μc/1 ul) transport studies. The culture incubated for various periods of time contained .5 μc of the labeled substance in .5 ml dextrose-free media or BSS. For the inhibition studies glucose, methyl-D-glucose, 2-deoxy-D-glucose, xylose and phlorizin in various concentrations were added to the labeled solution. In addition, the extracellular space was determined with ^{14}C sucrose or ^{14}C inulin. After the incubation, the medium was removed and the cultures were washed three times with BSS. Pooled samples (2-7 cultures) were either weighed or assayed for protein content (Lowry, O. H.), solubilized and processed for liquid scintillation counting.

Major Findings: The myelinating organotypic cerebellar cultures were composed of glial cells, large neurons and granular cells. The highest accumulations of ^{3}H 3-MG and ^{3}H 2-DG in the cerebellar explants were found after 30 minutes of incubation at pH 7 - 7.2. At this pH, the least diffusion of the tested substances occurred from the media into the cultures.
The Km that is the apparent concentration of half maximal uptake of 3-MG and 2-DG was 11 μM and 4 μM, respectively. Elimination of NaCl from the media did not significantly change the uptake of either ^{3}H 3-MG or ^{3}H 2-DG of the explants. The uptake of ^{3}H 3-MG was 44 mM/mg tissue from NaCl-free medium and 42 mM/mg tissue from regular medium, while the uptake of ^{3}H 2-DG was 123 mM/mg^{-1}P and 131 mM/mg^{-1}P by the cerebellar cultures in NaCl-free and normal medium, respectively. The results have been included in a manuscript submitted to the Journal of Neurobiology for publication (Renkawek, K., Spatz, M, Murray, M. R., and Klatzo, I.: Uptake of radiolabeled glucose analogues by organotypic cerebellar cultures).

Significance to Biomedical Research and the Program of the Institute: The cerebellar tissue culture is an excellent model for the study of transport across the cellular membrane elements without the interference of cerebral vessels. These investigations have been focused on creating the environmental and cytogenic conditions in tissue cultures similar to the one existing in normal and diseased brain in order to determine the cellular functions in many neurological disorders associated with ischemia, edema, neurotoxicity and others.

Proposed Course of the Project: This continued investigation will be concerned with the uptake of radiolabeled glucose analogues in cultures subjected to hypoxia, and an excess of metabolites which are present in the brain tissue subjected to ischemia. This model will also be used for defining the uptake of amino acids under normal and pathological conditions.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02084-04 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Properties of cerebral capillaries in organotypic cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Bubis	Professor Pathol.	Chaim Sheba Med. Ctr., Israel
OTHER:	M. Spatz	Head, Section on Neurocytol.	LNNS NINCDS
	M. R. Murray	Research Biologist	LNNS NINCDS
	K. Renkawek	Assoc. Professor	Inst. Neuropathol., Warsaw
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

The Chaim Sheba Medical Center, Tel Hashomer, Israel
Institute of Neuropathology, Polish Academy of Sciences, Warsaw, Poland

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ultrastructurally, the capillaries cultivated in the organotypic cerebellar explants showed the same characteristic tight junction between the endothelial cells as the one seen in situ. The alkaline phosphatase activity was confined to the plasma membrane.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The concept of blood-brain barrier (BBB) is essentially functional. Nevertheless, realization of the function to admit or exclude certain types of substances to the cerebral tissue must be based, at least in part, on morphological and histochemical factors of the capillaries. It occurred to us that the organotypic cerebellar and pia arachnoid cultures might be suitable for the isolation, identification and histochemical studies of the BBB capillaries in order to define and differentiate their function from the capillaries of tissue unrelated to the BBB system.

Methods Employed: The Maximow technique was used for cultivation of capillaries from cerebellar or leptomeningeal explants. The cerebellum was stripped from meninges and each one sectioned into small fragments. The cerebellar tissue was placed while the meninges were spread in collagen-coated cover slips. The cultures were fed and washed twice weekly. The nutrient medium was composed of equal parts of balanced salt solution (BSS), fetal calf serum, some serum ultrafiltrate supplemented with 300 mg% of glucose. The outgrowth of cells and the formation of capillaries have been observed by light and phase microscopy for over two months. Representative cultures were photographed living, with phase and subsequently fixed in glutaraldehyde and post-fixed with osmium for plain ultrastructural examination. Another group of similar or sister cultures was incubated for alkaline phosphatase prior to the preparation of the blocks for electron microscopy.

Major Findings: This continuing investigation of enzymatic and morphologic properties of endothelial cells in cerebellar and leptomeningeal organotypic cultures has been extended to the ultrastructural level. So far, a pilot study has been done showing the same characteristic features of the cerebellar capillaries grown in culture as in situ. The tight junctions can be identified between the endothelial cells. The activity of the alkaline phosphatase was confined to the plasma membrane.

This project has been continued in collaboration with Dr. Bubis of the Chaim Sheba Medical Center, Tel Hashomer, Israel. Due to technical difficulties in the shipment of the specimens, a temporary delay of the investigation has taken place.

Significance to Biomedical Research and the Program of the Institute: The cerebellar and/or leptomeningeal organotypic cultures are a suitable model for studying the function of the endothelial cells and their role in transport and/or metabolism of the brain in health and disease. Such information is of great importance for the understanding of many neurological disease processes, especially the cerebrovascular disorders.

Proposed Course of the Project: This model will be utilized also for the study of other capillary enzymes localization in these organotypic cultures by electron microscopy. Furthermore, the cultivated microvessels will be examined by scanning microscope and their properties compared with capillaries of organs not involved in the blood-brain barrier.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02085-04 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cerebrovascular Permeability to Peroxidase in Ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Westergaard	Assoc. Prof. Anatomy	Univ.	Copenhagen
OTHER:	M. Spatz	Head, Section on Neurocytobiol.	LNNS	NINCDS
	K.G. Go	Assoc, Prof. Neurosurgery	Univ.	Groningen
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS	NINCDS

COOPERATING UNITS (if any)
Anatomy Dept., University of Copenhagen, Copenhagen, Denmark
Dept. of Neurosurgery, University of Groningen, Groningen, The Netherlands

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SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: .35	PROFESSIONAL: .1	OTHER: .25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF 'RK (200 words or less - underline keywords)

Mongolian gerbils subjected to unilateral cerebral ischemia showed an increased vesicular transport of peroxidase in the cerebral arterioles, venules and capillaries in the affected cerebral hemisphere. The amount of transferred peroxidase across the microvessels increased with duration of cerebral blood supply deprivation, which never resulted in degeneration of the endothelial cells in the brain vessels. The intercellular spaces between the endothelial cells were free of peroxidase from the lumen to the first abluminal tight junction. This project has been completed.

Project Description:

Objectives: Cerebral infarctions occur in 30-50% of gerbils subjected to the occlusion of a single common carotid artery (Ito, U., Spatz, M., Walker, J.T., Jr., and Klatzo, I., Acta Neuropath. 32: 209-223, 1975). In the animals with cerebral injury, an augmented facilitated transport of glucose analogues was observed after 6 hours, while an increased passive diffusion of glucose occurred after 18 hours of carotid artery clipping. In gerbils without apparent cerebral damage, only the facilitated transport of glucose analogues was found to be increased irrespective of the duration of carotid artery occlusion. These findings suggested that the observed change and the type of glucose transport may depend on the integrity of the cerebral capillaries (Spatz, M., Go, K. G., and Klatzo, I. The effect of ischemia on the brain uptake of ^{14}C glucose analogues and ^{14}C sucrose. In Cervos-Navarro, J. (Ed.): Pathology of Cerebral Microcirculation. Berlin, West Germany, Walter de Gruyter & Co., 1974, pp. 361-366). Thus, the present investigation was designed to evaluate the status of the cerebral capillaries by electron microscopy in ischemic gerbils.

Methods Employed: Several groups of gerbils submitted to various periods of left common carotid artery occlusion and 1 hour release were injected with horseradish peroxidase (10 mg/100 g body weight) as the blood-brain barrier (BBB) tracer. The horseradish peroxidase was allowed to circulate for 5 minutes and thereafter the brains were perfused with paraformaldehyde. Selective tissue sections of the cerebral hemisphere were processed for light and electron microscopy from animals with and without cerebral symptoms.

Major Findings: In the post-ischemic period after 3 hours of unilateral common carotid artery occlusion, the affected cerebral hemishphere of the gerbils showed a slight increased swelling of the astroglial processes with an increased permeability of arterioles, venules and capillaries to the horseradish peroxidase tracer. These changes were more pronounced after 6 hours of ischemia, but the plasma membrane of the astroglial cells adjacent to the vessels were not permeable to the peroxidase. The occlusions of 18 hours severely damaged the plasma membranes of the neuroglial cells and the peroxidase was observed in the cytoplasm of the astroglial endfeet and in the cytoplasm of other cells. No endothelial cell damage was seen at any time, although the number of peroxidase vesicles in the endothelial cells progressively increased with the duration of ischemia. Part of this work was presented at the International Symposium on "Pathophysiological, Biochemical and Morphological Aspects of Cerebral Ischemia and Arterial Hypertension", Sept. 18-20, 1975, Warsaw, Poland.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the nature and sequence of vascular and parenchymatous ischemic brain injury is of major importance in the understanding of the cerebrovascular disease process. The exploration of the established animal model system for cerebral ischemia should be helpful and useful in the comprehension and treatment of this disease process in man.

Proposed Course of Project: This project has been completed.

Publications:

Westergaard, E., Go, K. G., Klatzo, I. and Spatz, M.: Increased permeability of cerebral vessels to horseradish peroxidase induced by ischemic in Mongolian gerbils. Acta Neuropath. 35: 307-325, 1976.

Westergaard, E., Go, K.C., Klatzo, I. and Spatz, M.: Enhanced vesicular transport of horseradish peroxidase across cerebral vessels induced by ischemia. In: International Symposium on Pathophysiological Biochemical and Morphological Aspects of Cerebral Ischemia and Arterial Hypertension, Warsaw (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02165-03 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Correlation of ^{3}H isoleucine uptake in pia arachnoid with culture of fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
OTHER:	M. R. Murray	Research Biologist	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.4	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF [illegible] (200 words or less - underline keywords)

The correlative studies of ^{3}H isoleucine uptake in pia arachnoid explants and in the fibroblastic culture of skin have shown that the uptake is saturable and could be cross inhibited by L leucine and cycloleucine in both types of cultures. However, the pia arachnoid was found to be different from the fibroblasts since it (1) couldn't accumulate the amino acid as did the fibroblasts and (2) couldn't be cross inhibited by D leucine, thus displaying stereospecificity for the L form of the amino acid. Therefore, the pia arachnoid may represent a site for regulation of amino acid transport in the central nervous system.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 02165-03 LNNS

Project Description:

Objectives: Facilitated carrier mediated glucose transport was described in organotypic pia arachnoid cultures recently (Spatz, M., Renkawek, K., Murray, M.R., and Klatzo, I.: Brain Res. 100: 710-715, 1975). This is a continuation study of pia arachnoid cultures in order to characterize its functions under normal and pathological conditions.

Methods Employed: Pia arachnoid explants from newborn rats were cultivated in the Maximow double cover slip depression slide assembly according to the technique of Allerand and Murray, 1968. Fetal fibroblasts were grown in MEM Eagle's media containing 1 mM glutamine and 10% fetal calf serum. Radiolabeled uptake of isoleucine and the non-metabolizable cycloleucine were studied in 14 day old cultures. The experiments were performed in triplicate in saline (BSS) washed cultures. They were incubated in .05 ml of BSS containing .5 μc of the ^3H labeled isoleucine or cycloleucine and .025 μc of ^{14}C inulin at pH 7.4 for 2-15 minutes. Various concentrations of either unlabeled cycloleucine or isoleucine or L-leucine or D-leucine were added to the incubation media for the inhibition studies. Thereafter, the cultures were washed, extracted with 10% trichloracetic acid and the amount of isotope was determined by liquid scintillation counter. The protein was assayed according to Lowry et al. technique (J. Biol. Chem. 193: 265-275, 1951).

Major Findings: The pia arachnoid uptake of ^3H isoleucine was lower than in the fibroblastic cultures. After 1 minute of incubation in the ^3H isoleucine, the concentration of the isotope in the pia arachnoid explant didn't exceed the concentration of ^3H isoleucine in the medium, while the fibroblastic culture's uptake was about 12 times higher than the level of ^3H isoleucine in the medium. A linear increase of ^3H isoleucine took place for 1-5 minutes in both cultures but the concentration of the isotope was always lower in the pia arachnoid than in the fibroblasts. Moreover, in both cultures the uptake of ^3H isoleucine was saturable with addition of unlabeled isoleucine in increasing concentration to the incubating medium containing the labeled substance. The uptake could be also cross inhibited by addition of unlabeled L-leucine and cycloleucine. However, D-leucine affected only the isoleucine uptake of the fibroblasts but not the pia arachnoid cultures. All the kinetic data are being analyzed in preparation of a manuscript.

Significance to Biomedical Research and the Program of the Institute: The pia arachnoid explant due to its relatively simple composition of pial membrane and vessels is an excellent model for the investigation of its function. The determination and evaluations of the uptake of various substances by pia arachnoid will permit us to assess the permeability of these structures. Thus, these studies will be helpful in defining its properties and possible role in the relation to blood and spinal fluid in the normal and diseased state.

Proposed Course of the Project: The continued investigations will be concerned with defining and eliciting the factors responsible for the amino acid uptake under normal conditions. Therefore, the kinetics and the influence of electrolytes, metabolites, and drugs will be determined in both types of cultures. Thereafter, this model will be used to study the amino acids uptake under pathological conditions.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02166-03 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Synaptosomal 2-deoxy-D-(^{3}H)-glucose uptake in cerebral ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
OTHER:	B.B. Mrsulja	Professor of Biochem.	Inst. Biochem. Belgrade
	B.J. Mrsulja	Research Physician	Inst. Biol. Belgrade
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
Institutes of Biochem. and Biology, Faculty of Medicine, Belgrade, Yugoslavia

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was temporarily discontinued for the fiscal year 1976-1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02196-02 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Isolation of cerebral microvessels

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Mrsulja	Professor Biochem.	Inst. Biochem., Belgrade
OTHER:	B. J. Mrsulja	Research Physician	Inst. Biol., Belgrade
	T. Fujimoto	Visiting Scientist	Univ. W. Ontario
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

Institutes of Biochemistry and Biology, Faculty of Medicine, Belgrade, Yugoslavia
University of Western Ontario, London, Ontario, Canada

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Laboratory of Neuropathology and Neuroanatomical Sciences

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.95	.5	.45

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A simple method was developed to isolate enzymatic and metabolic active fraction of cerebral microvessels from nonvascular brain tissue which showed a saturable uptake of 2-deoxy-D-(^{3}H) glucose. The Km for ^{3}H 2-deoxy-D-glucose was 0.1 mM and the uptake was inhibited by the non-metabolizable 3-0-methyl-D-glucose and the inhibitors of carrier transport phlorizin and phloretin. The project is completed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Few different procedures have been described for the isolation of brain capillaries recently. However, the methods were cumbersome requiring repeated use of nylon cloth sieve or glass beads columns for elimination of nonvascular cerebral tissue in order to obtain relatively pure fractions of capillaries (Goldstein, G. Y., Wolinsky, J. S., and Diamond, I., J. Neurochem. 25: 715-717, 1975; Joo, F., and Karunshina, I., Cytobios 8: 41-48, 1975; and Orlowski, M., Sessa, G., and Green, J. P., Science 184: 66-68, 1974). The purpose of this study was to modify the technique for isolation of pure cerebral capillary fractions which could be used for transport and metabolic investigations of cerebral microvessels in normal and pathological conditions.

Methods Employed: Rabbit cerebral tissue (forebrain freed from pia arachnoid membrane) was homogenized in a cold Ringer's solution containing 1% bovine serum albumin at 10 mM Hepes. The homogenate was centrifuged twice at 1000 g for 10 minutes. Then the resuspended pellet was layered over a sucrose gradient and ultracentrifuged at 58,000 g for 30 minutes. The pellet was examined histologically, and histochemically and the capillary uptake of 2-deoxy-D-glucose (2-DG) was determined using radioactive hexose.

Major Findings: The isolated microvessels revealed histologically a predominance of strands, ribbons and coils of tubular structures comprised of single layer of cells devoid of smooth muscle containing reticulum fibers consistent with capillaries. Histochemically, an intense activity of γ-glutamyl-transpeptidase and butyryl-cholinesterase was demonstrated in the endothelial cells. The specific uptake of ^3H 2-DG was saturable with increased substrate concentrations. The uptake of this partially metabolizable hexose could be inhibited up to 80% with progressively increased unlabeled non-metabolizable 3-0-methyl-D-glucose, 1 mM phlorizin and 1 mM phloretin.

Significance to Biomedical Research and the Program of the Institute: The availability of an easy technique for the separation of cerebral capillaries from the nonvascular tissue provides a good method for the study of transport metabolic phenomena occurring on the blood-brain barrier level in normal and pathologic conditions, for example, in ischemia.

Proposed Course of the Project: The project of cerebral microvessels isolation is completed but this model system will be used for the study of glucose, amino acids and biogenic amines uptake in microvessels obtained from the brains subjected to altered pO_2 saturation, and pCO_2 tension, ischemia and recovery.

Publications:

Mrsulja, B. B., Mrsulja, B. J., Fujimoto, T., Klatzo, I. and Spatz, M.: Isolation of brain capillaries: A simplified technique. Brain Res. 110: 361-365, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02197-02 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Demonstration of ATPase in cerebellar cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
OTHER:	K. Renkawek	Assoc. Prof.	Inst. Neuropathol.,Warsaw
	M.R. Murray	Res. Bio.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

Institute of Neuropathology, Polish Academy of Sciences
Warsaw, Poland

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been discontinued for the present time.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02198-02 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Ischemic and postischemic effect on 2-deoxy-D (^{3}H) glucose uptake in cerebral capillaries.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
OTHER:	B. B. Mrsulja	Professor Biochem.	Inst. Biochem. Belgrade
	D. Micic	Visiting Fellow	LNNS NINCDS
	B. J. Mrsulja	Research Physician	Inst. Biol. Belgrade
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
Institutes of Biology and Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.3	.55

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

2-Deoxy-D-(^{3}H) glucose uptake was studied in isolated cerebral capillaries obtained from gerbils subjected to bilateral common carotid artery occlusion with and without release. Both the ischemia and the postischemic recirculation of the cerebral blood flow affected the capillary function as was evident by the reduction and increase of ^{3}H 2-DG uptake respectively. Both processes were transient and dependent on the duration of the experimental period.

Project Description:

Objectives: In previous studies we observed an increased uptake of 2-deoxy-D-(^3H) glucose (^3H 2-DG) in gerbils after short and long term ischemia (Spatz et al., in "Pathology of Cerebral Microcirculation," 361, 1976, and in "Transport Phenomena in Nervous System," 479, 1976). These noted changes could have been the result of an altered transport at the capillary and/or glial and neuronal sites. Since the isolation of metabolically active (Mrsulja et al., Brain Res. 110, 361, 1977) cerebral capillaries became possible, we thought that such a fraction would be of great help in clarifying some aspects of transport processes occurring under these conditions.

Methods Employed: Groups of Mongolian gerbils were anesthetized intraperitoneally with pentobarbital (3.5 mg/100 g) 30 min prior to bilateral common carotid artery occlusion for 1, 3 and 6 min. The cerebral circulation was reestablished for 1-15 min and 1-5 hrs in animals subjected to 6 min ischemia only. Thereafter the animals were killed by decapitation and the cerebral microvessels were separated from nonvascular tissue by fractionation (Mrsulja, et al., Brain Res. 110, 361, 1976). Then the obtained cerebral capillary fraction was used for ^3H 2-DG uptake and inhibition studies.

Major Findings: A significant decrease in specific capillary uptake of ^3H 2-DG (42.2 ± 3.6 SEM) as per cent of control uptake was observed in animals subjected to bilateral carotid artery clipping for 6 min but not for 1 and 3 min. Full recovery of the capillary function occurred after arterial clip release from 5-15 min. Thereafter a progressively increased ^3H 2-DG uptake of cerebral capillaries was seen up to 1 hour post release. This increased uptake of ^3H 2-DG could be inhibited to the same degree as in control capillaries by nonmetabolizable 3-O methyl-D-glucose. The return to normal uptake was found in cerebral capillaries obtained from animals whose cerebral circulation was reestablished for 5 hrs after 6 min of bilateral common carotid artery occlusion.

Significance to Biomedical Research and the Program of the Institute: Based on our investigation, the cerebral capillaries are useful for the study of some parameter brain transport phenomena occurring in both physiological and pathological conditions. The knowledge of the functional state of cerebral capillaries is extremely important, since it may either be responsible for many metabolic changes occurring the brain and/or it may reflect the altered metabolic state of the brain in many disease processes.

Proposed Course of the Project: Similar model will be used for the study of amino acid capillary uptake as well as release in ischemic and postischemic gerbils.

Publications:

Spatz, M., Mrsulja, B. B., Micic, D., Mrsulja, B. J. and Klatzo, I.: Ischemic and post-ischemic effects on the 2-deoxy-D-[^3H] glucose uptake in cerebral capillaries. Brain Res. 120: 141-145, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02274-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Brain serotonin after experimental vascular occlusion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Mrsulja	Professor Biochem.	Inst. Biochem., Belgrade
OTHER:	B. J. Mrsulja	Research Physician	Inst. Biol., Belgrade
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

Institutes of Biology and Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.2	.25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINOR ☐ (a2) INTERVIEWS

SUMMARY OF K (200 words or less - underline keywords)

Unilateral common carotid artery occlusion in gerbils produced a decreased level of 5-HT and accumulation of 5-HIAA in the ischemic cerebral cortex. The reduction of 5-HT was aggravated by pretreatment with PCPA the inhibitor of tryptophan hydroxylase and pargyline the inhibitor of monoamine oxidase suggestive of decreased 5-HT synthesis and degradation, respectively, occurring in ischemia. Moreover the inhibition of 5-HIAA out-transport from brain was indicated by the more pronounced accumulation of 5-HIAA probenecid pretreated ischemia than the ischemic animals which 5-HT levels were low in the cerebral cortex. This project is completed.

Project Description:

Objectives: It has been suggested recently by Welch et al. (J. Neurol. Neurosurg. Psychiat. 36: 724, 1973) that the abnormal 5-hydroxytryptamine release might be responsible for the progression of cerebral infarction. Since a decrease of 5-HT content of cerebral cortex was observed after 3 hrs of ischemia (Brain Res. 98: 394, 1975), we undertook to characterize the reduction of 5-HT using pharmacologic methods for the study of 5-HT turnover.

Methods Employed: Cerebral ischemia was produced in Mongolian gerbils by unilateral occlusion of common carotid artery for 3 hours. Pargyline (75 mg/kg), p-chlophenylalanine (500 mg/kg) or probenecid (200 mg/kg) were injected intraperitoneally 2 hours prior to arterial occlusion. Sham operated animals injected with saline served as controls. The concentration of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were analyzed spectrophotofluorometrically (J. Neurochem. 20: 1777, 1975).

Major Findings: Deprivation of blood supply to the brain produces reduction of 5-HT concentration in the cerebral cortex [(150 ng/g) - control 200 ng/g]. Additional decrease of 5-HT levels occurred in ischemic animals after inhibition of tryptophan hydroxylase, the rate limiting enzyme in monoamine synthesis, by means of PCPA (50 ng/g) and reduced concentration of 5-HT in the brain was observed in ischemic gerbils treated with pargyline, an inhibitor of monoamine oxidase, the enzyme catalyzing the formation of 5-HIAA from 5-HT [(220 ng/g) - control pargyline treated 380 ng/g]. Moreover 5-HIAA concentration increased in the brain following vascular occlusion [(710 ng/g - control 470 ng/g]. The accumulation of this major 5-HT metabolite was observed even when its formation was inhibited by means of pargyline or 5-HT was inhibited by means of PCPA. In addition a large accumulation of 5-HIAA was obtained when vascular occlusion was produced in animals treated with probenecid [(900 ng/g) - controls 740 ng/g].

Significance to Biomedical Research and the Program of the Institute: These results suggest that the reduction of 5-HT in the brain of the ischemic gerbils is due to increased release of this monoamine, since the rates of its synthesis and degradation are depressed after vascular occlusion. Furthermore, our results indicate that essentially no available 5-HT for 5-HIAA is present after vascular occlusion, since both synthesis and degradation of 5-HT are depressed under these conditions. Therefore the accumulation of 5-HIAA in ischemic gerbils may be due to a decreased out-transport from the brain and spinal fluid since the accumulation of 5-HIAA was greater in probnecid pretreated ischemic than in the ischemic animals only. These studies contribute greatly toward the basic understanding of the fate of neurotransmitters in cerebral ischemia. Moreover they provide another dimension for elucidating possible factors contributing to the process of the cerebrovascular disorders, which may have therapeutic implications.

Proposed Course of the Project: Completed.

Publications:

Mrsulja, B. B., Mrsulja, B. J., Spatz, M. and Klatzo, I.: Brain serotonin after experimental vascular occlusion. Neurology 26: 785-787, 1976.

Mrsulja, B. B., Mrsulja, B. J., Spatz, M. and Klatzo, I.: Monoamines in cerebral ischemia in relation to brain edema. In: Dynamics of Brain Edema, Pappius, H. M., and Feindel, W. (Eds.). Berlin Heidelberg, Springer-Verlag, 1976, pp. 187-192.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02275-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cerebral capillary endothelial cultures.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
OTHER:	D. Micic	Visiting Fellow	LNNS NINCDS
	M. Murray	Research Biologist	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: .6	PROFESSIONAL: .3	OTHER: .3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In a pilot study, a few cerebral capillary endothelial cell cultures have been established from isolated cerebral capillaries of 2 day old rats. The cells have been growing slowly as sheets of elongated cells (with plump nuclei) forming bands and occasionally loops.

Project Description:

Objectives: The availability of simple technique for isolation of cerebral capillaries (Mrsulja, B. B., Mrsulja, B. J., Klatzo, I., and Spatz, M., Brain Res. 110: 361-365, 1976) provided the impetus for establishment of cerebral capillaries as an organotypic and dissociated cell culture for morphological, histochemical and biochemical studies under normal and pathologic conditions.

Methods Employed: The cerebral capillaries were separated from the non-vascular tissue of 2 day old rats by homogenization, centrifugation and sucrose gradient under sterile conditions. The cellular pellet was washed 4 times in Simms' balanced solution (BSS) containing antibiotics for a 30 minute period, centrifuged at 1000 rpm and resuspended in fresh BSS. Thereafter the capillaries were suspended in 25 ml Trypsin-Versene solution and dissociated for 30 minutes. The process was repeated after 10 minutes of recentrifugation at 1000 rpm. The cultures kept in T-60 flasks or in Petri dishes have been cultivated in a mixture of 199 medium containing 30% fetal calf serum, amino acids,MEM vitamin solution and antibiotics for 1 week. Thereafter the cultures are fed twice a week with the same medium but with a reduced content of fetal calf serum (20%).

Major Findings: This project was undertaken only a few months ago, but we have been successful in establishing capillary endothelial cultures from both the organotypic explant and dissociated cells. These cells grow very slowly as sheets of elongated cells with plump nuclei forming bands and occasionally an attempt at loop formation was seen in these cultures. So far the oldest culture is 2 months old and we still don't have enough material for the investigation on the properties of these cells.

Significance for Biomedical Research and the Program of the Institute: The establishment of cerebral capillary endothelial cell cultures will provide a pure cell line which will be useful for the investigation of cerebral endothelial cells in the living state without the influence of any other cells. Thus, the function of cerebral capillary endothelium as compared to endothelium derived for capillaries not belonging to the blood-brain barrier (BBB) system can be characterized under normal and pathologic conditions. This approach will also add another dimension for the studies related to the BBB permeability.

Proposed Course of the Project: The primary objective of this project has been to obtain an easily reproducible endothelial cell line which will provide sufficient material for morphological and histochemical investigations of the cerebral capillary endothelial properties in the living state as compared to the one in situ. Thereafter various functional studies of these cells and the ones derived from capillaries of other organs will be studied using histochemical, immunological and radioautographic techniques.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02276-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Ischemic and postischemic effect on the activity of cytochrome oxidase and acetylcholinesterase in Mongolian gerbils

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Micic	Visiting Fellow	LNNS NINCDS
OTHER:	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NIH, NINCDS, Bethesda, Maryland 20014

TOTAL MANYEARS: .3	PROFESSIONAL: .3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cytochrome oxidase and acetylcholinesterase activities were assayed during short and long term ischemia as well as in various recovery periods after 1 hour of cerebral blood flow deprivation. The cytochrome oxidase activity was affected only in the recovery period after one hour ischemia and after 5 hours of continuous ischemia. At that time the activity of cytochrome oxidase was significantly reduced when compared to the controls. In contrast, no significant changes in the activity of acetylcholinesterase were observed in the experimental as compared to control gerbils.

Project No. Z01 NS 02276-01 LNNS

Project Description:

Objectives: Cerebral ischemia appears firstly to affect the mitochondria, then the endoplasmic reticulum and ribosomes (Hagen et al., 1960, Hills, 1964, and Clendenon et al., 1971). However our recent studies have shown that one of the mitochondrial enzymes the monoamino oxidase is not affected during short term ischemia but in the recovery period or after prolonged continuous ischemia of 5 hours. Therefore we investigated another mitochondrial enzyme the cytochrome oxidase, which is involved in electron transport system and correlated with the behavior of synaptosomal enzyme, acetylcholinesterase activity in cerebral ischemia and postischemia of gerbils.

Methods Employed: Several groups of anesthetized animals (pentobarbital 20 mg/kg i.p.) were subjected to unilateral carotid artery occlusion for 1 hour and various periods of release or continuous occlusion of 1-5 hours. The mitochondrial cytochrome oxidase activity was assayed (Hess, H. and Pope, A., *J. Biol. Chem.* 204: 295, 1953).

The synaptosome activity of acetylcholinesterase was determined by the Ellman spectrophotometric method (*Biochem. Pharmac.* 7: 88, 1961).

Major Findings: The cytochrome oxidase activity was significantly reduced at 20 hrs and remained low at 72 hrs and 1 week after the reestablishment of cerebral blood circulation in 1 hr occluded gerbils. A significantly decreased activity of this enzyme was also observed after 5 hrs of continuous cerebral flow deprivation. In contrast the acetylcholinesterase activity was not affected by cerebral ischemia or postischemic cerebral recirculation of blood.

Significance to Biomedical Research and the Program of the Institute: These studies indicate that the cytochrome activity is affected the same way as previously studied (mitochondrial enzyme) MAO by cerebral ischemia and postischemia. The reduction of this enzyme occurs only after long term continuous ischemia or in the recovery period from short term ischemia. The absence of changes in the acetylcholinesterase activity indicates that the deprivation and reestablishment of cerebral blood circulation has no effect on the enzyme responsible for the metabolism of cholinergic neurotransmitter.

Proposed Course of the Project: This project is almost completed except for the evaluation of the enzyme activities in relationship to the so-called "maturation phenomenon."

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02277-01 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemistry of cerebral microvessels: Enzymic changes in ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Mrsulja	Professor Biochem.	Inst. Biochem., Belgrade
OTHER:	B. M. Djuricic	Postgraduate Fellow	Inst. Biochem., Belgrade
	B. J. Mrsulja	Research Physician	Inst. Biol., Belgrade
	L. Rogac	Postgraduate Fellow	Inst. Biochem., Belgrade
	I. Klatzo	Chief, Neuropath. Neuroanat. Sci.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS

COOPERATING UNITS (if any)

Institutes of Biology and Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINOR ☐ (a2) INTERVIEWS

SUMMARY OF K (200 words or less - underline keywords)

The activity of several enzymes has been investigated in isolated cerebral microvessels versus total brain homogenate obtained from normal and ischemic gerbils. The preliminary studies suggest that the capillary enzymes are affected by ischemia prior to the brain parenchyma.

Project No. Z01 NS 02277-01 LNNS

Project Description:

Objectives: The simple technique for isolation of cerebral capillaries (Mrsulja et al., Brain Res. 110: 361, 1976) provides an opportunity to study metabolic events occurring in the microvessels under normal and pathologic conditions.

Methods Employed: Several enzyme activities were assayed in the cerebral capillaries versus total brain homogenate isolated from normal and ischemic gerbils. The cerebral ischemia was produced by bilateral common carotid artery occlusion for 1-9 minutes.

Major Findings: In normal animals glutamyl transpeptidase, butyrylcholinesterase, alkaline phosphatase and leucine aminopeptidase are found in the capillary fraction, while enzymes of energy and amino acid metabolism as well as acetylcholinesterase are present mainly in the brain parenchyma. In bilateral ischemia the following enzymes were found to be reduced: HK, PK, LDH, AChE, BuChE and ATPase, whole PFK, G-6-Pase and gamma-GT activities are increased. There was no change of G-6-PDH, AChE and BuChE activities in the brain parenchyma, although NAD-dependent isocitive dehydrogenase and HK activities were decreased.

Significance to Biomedical Research and the Program of the Institute: The metabolic studies of microvessels isolated from brain may lead to the understanding of factors regulating BBB permeability in normal and pathologic conditions. These events are of great importance in order to elucidate many disease processes and to select the best therapeutic approaches.

Proposed Course of the Project: The metabolic study of the microvessels will be extended to the postischemic period after this project is completed.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02278-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of anoxia on 2-deoxy-D (^{3}H) glucose uptake in isolated cerebral capillaries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Micic	Visiting Fellow	LNNS NINCDS
OTHER:	J. Micic	Guest Worker	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiology	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.5	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

2-Deoxy-D (^{3}H) glucose (^{3}H 2-DG) uptake was studied in isolated normal cerebral capillaries subjected to various gas mixtures during incubation. Under 100% N_2 atmosphere, the specific ^{3}H 2-DG capillary uptake was markedly decreased but could be recovered by substituting the N_2 gas for oxygen or normal air. Moreover the addition of free fatty acid serum albumin prevented the reduction of ^{3}H 2-DG capillary uptake under anaerobic conditions.

Project Description:

Objectives: A decreased 2-deoxy-D-(^{3}H) glucose (^{3}H 2-DG) uptake was observed in the capillary fraction isolated from brains of gerbils subjected to bilateral cerebral ischemia (Spatz et al., Brain Res. 120: 141, 1977). In order to clarify some aspects of the pathophysiological mechanism responsible for that phenomenon, we investigated the effect of O_2 deprivation on the ^{3}H 2-DG uptake in the cerebral capillary fraction separated from the nonvascular normal brain tissue.

Methods Employed: The procedures for the capillary isolation and ^{3}H 2-DG studies were the same as the ones described previously (Mrsulja et al., Brain Res. 110: 361, 1976). However 26 mM K phosphate buffer containing 146 mM of sucrose was used instead of Ringer solution for the incubation in the atmosphere of various gases.

Major Findings: A markedly reduced (75%) specific ^{3}H 2-DG uptake was found in the capillaries incubated in nitrogen atmosphere as compared with the one exposed to normal air or 100% oxygen or 95% N_2 and 5% O_2. The decreased capillary ^{3}H 2-DG uptake could be recovered by substituting the atmosphere with normal air or oxygen after 7.5-15 minutes exposure to N_2 gas. The anoxic inhibition of ^{3}H 2-DG uptake couldn't be restored by the addition of either energy phosphate metabolites or mono-, bivalent ions or various free fatty acids to the incubation buffer. Only the addition of essentially fatty acid free serum albumin prevented the ^{3}H 2-DG reduction of ^{3}H 2-DG capillary uptake under anaerobic conditions.

Significance to Biomedical Research and the Program of the Institute: The preliminary results of this investigation are of great importance for the understanding of glucose transport from blood to brain since the transfer of this primary brain nutrient is oxygen dependent, which has been previously denied. Moreover it appears that the transport of glucose may be closely related to free fatty acid metabolism, which was not considered previously. The basic understanding of the cerebral capillary function may help in elucidating the mechanism of cerebrovascular disease and possibly the altered function of other neurological diseases.

Proposed Course of the Project: This project is in progress and in order to complete it, an attempt will be made to define the relationship of oxygen-dependent glucose uptake and the free fatty acid metabolism.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02279-01 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Electrolyte changes in ischemic cerebral edema

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Pappius	Assoc. Prof. Neurochem.	Montreal Neurol. Inst.
OTHER:	T. Fujimoto	Visiting Scientist	Univ. W. Ontario
	K. Nishimoto	Visiting Fellow	LNNS NINCDS
	K. G. Go	Assoc. Prof. Neurosurg.	Univ. Groningen
	I. Klatzo	Chief, Neuropath. Neuroanat. Sci.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS

COOPERATING UNITS (if any)
Montreal Neurological Institute, Montreal, Quebec, Canada
University of Western Ontario, London, Ontario, Canada
Department of Neurosurgery, University of Groningen, Groningen, The Netherlands

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The analysis of the electrolyte brain content in ischemic cerebral edema has been in progress. The preliminary results suggest a biphasic alteration of electrolyte levels in the ischemic brain corresponding to the changes in water content of the brain. The peak of the reduced K^+ content and the elevation of Na^+ level in the affected brain coincide with the occurrence of the increased BBB permeability to proteins.

Project Description:

Objectives: In human cerebral ischemia brain edema is considered to be an important factor in causing mortality (Shaw, C., Alvord, E., and Berry, R., Arch. Neurol. 1: 161-177, 1959). Experimentally, cerebral ischemia can be easily produced in Mongolian gerbils by ligation of a single common carotid artery (Levine, S., Payan, H., Exp. Neurol. 16: 252-255, 1966; Kohn, C., Arch. Path. 69: 544-553, 1972; Ito et al., Acta. Neuropath. 32: 209-223, 1975). In our recent studies a development of biphasic ischemic cerebral edema was observed after a short term ischemia of 1 hour duration (Spatz, M., Fujimoto, T., Go, G. K., in Dynamics of Brain Edema, Berlin Heidelberg, Springer-Verlag, 1976, pp. 181-186). The present investigation has been concerned with the evaluation of cerebral electrolyte levels under the same conditions in order to elucidate further the pathophysiological mechanism responsible for the development of ischemic edema.

Methods Employed: Several groups of adult gerbils were subjected to unilateral clipping and clip release of the left common carotid artery for various periods of time. Only the gerbils with definite cerebral symptoms were selected for this study and sham operated animals were used as controls. The cerebral water content was determined by wet and dry weight. The sodium and potassium were analyzed by flame photometry.

Major Findings: This project is in progress but the preliminary results demonstrate a biphasic alteration of electrolyte levels in the ischemic brain corresponding to water content of the brain. After 1 hour of occlusion, the concentration of K$^+$ is already decreased while Na$^+$ level is increased in the affected hemisphere as compared to controls (experimental K$^+$ 369 ± 21.9 meg/kg dry weight, Na$^+$ 48.7 ± 5.9 meg/kg wet weight; controls K$^+$ 432 ± 16.7 meg/kg dry weight and Na$^+$ 40.3 ± 2.5 meg/kg wet weight). Both the K$^+$ and Na$^+$ concentrations appear to return to normal at 5 hrs but the peak of secondary alterations occurs at 10 hrs after release of 1 hour occlusion. At this time the greatest reduction of K$^+$ and elevation of Na$^+$ level is seen in the ischemic hemisphere as compared to controls (K$^+$ 339 meg/kg dry weight, Na$^+$ 74.8 meg/kg wet weight; K$^+$ 432 meg/kg dry weight, Na$^+$ 40.3 meg/kg wet weight, respectively). These changes are taking place at the same time as an increased BBB permeability to albumin and substances of similar molecular size (Spatz et al., in Dynamics of Cerebral Edema, Berlin Heidelberg, Springer-Verlag, 1976, pp. 181-186). One week after the release of occlusion a reversibility of the electrolyte change is seen in about 50% of cases.

Significance to Biomedical Research and the Program of the Institute: Cerebral edema occurs as one of the major complications of many neurological disorders such as ischemia, trauma, tumors, chemical poisoning, and others. The basic understanding of the type of edema and its development is very crucial for the clinician who is faced not only with the diagnosis, but with the appropriate selection of treatment. Thus, various investigations of this problem are essential for finding the factor or factors responsible for the occurrence of cerebral edema and its treatment.

Proposed Course of the Project: To correlate the level of electrolytes in ischemic cerebral edema with the water content of the brain and with the BBB permeability under the same conditions in an attempt to define more closely the dynamics of this process.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02280-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The effect of cerebral ischemia and postischemia on monoamine oxidase activity (MAO).

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Micic	Visiting Fellow	LNNS NINCDS
OTHER:	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS: .25	PROFESSIONAL: .25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WC (200 words or less - underline keywords)

The effect of cerebral ischemia and postischemia on MAO activity was investigated in Mongolian gerbils. Ischemia of 1 hour duration doesn't alter the MAO activity until 20 hrs later when the reaction of MAO activity becomes apparent and persists for 1 week. In prolonged ischemia, the decrease in MAO activity is not seen prior to 5 hours of continuous unilateral common carotid artery occlusion.

PHS-6040
(Rev. 10-76)

Project No. 201 NS 02280-01 LNNS

Project Description:

Objectives: In our previous experiments we postulated that the monoamino oxidase activity couldn't be responsible for the observed ischemic and postischemic reduction of biogenic amines and accumulation of its metabolites in the brain (Mrsulja et al., Acta Neuropath. 36:1, 1976, and Brain Res. 98:388, 1975). In order to elucidate further the metabolic fate of these substances we investigated the cerebral monoamine oxidase (MAO) activity during ischemia and postischemia of Mongolian gerbils.

Methods Employed: Several groups of anesthetized animals (pentobarbital 20 mg/kg i.p.) were subjected to unilateral carotid artery occlusion for 1 hour and various periods of release or to continuous occlusion for 1-5 hrs. The mitochondrial MAO activity was assayed by microfluorimetric methods (Biochem. Pharmacol. 14: 1686, 1965).

Major Findings: The activity of MAO was significantly reduced in the hemisphere ipsilateral to 1 hour occlusion and release for 20 and 72 hrs and 1 week as compared to the contralateral to the one from control and sham operated animals (ischemia = 16.05 ± 1.35 (7), 12.99 ± 1.59 (7), 13.23 ± .76 (6) nmoles 4 HOQ/mg P/hr, respectively; control = 21.91 ± .79 (12) nmole 4 HOQ/mg P/hr). However, normal levels of the enzyme were found 4 weeks after the release of 1 hour occlusion. In the continuously ischemic gerbils the MAO activity was found to be only reduced at 5 hours [15.79 ± 1.0 (7) nmoles 4 HOQ/mg P/hr].

Significance to Biomedical Research and the Program of the Institute: The ischemic and postischemic reduction in MAO activity observed in these experiments substantiate our previous postulate that such alteration of the MAO couldn't be responsible for the decreased level of cerebral biogenic amines and accumulation of its metabolites in gerbils subjected to ischemia and recirculation of cerebral blood (Brain Res. 98: 388, 1975).

Proposed Course of Project: This project is almost completed except for the determination of MAO activity in relationship to the "maturaton phenomenon."

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02150-03 LNNS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Electronmicroscopic studies of the hippocampus in gerbils subjected to cerebral ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Bubis	Professor Pathol.	Chaim Sheba Med. Ctr., Israel
OTHER:	T. Fujimoto	Visiting Scientist	Univ. W. Ontario
	B. J. Mrsulja	Research Physician	Inst. Biol., Belgrade
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

Chaim Sheba Medical Center, Tel Hashomer, Israel
University of Western Ontario, London, Ontario, Canada
Institute of Biology, Faculty of Medicine, Belgrade, Yugoslavia

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was completed in Fiscal Year 1976. The resulting paper has since been published:

Bubis, J. J., Fujimoto, T., Ito, U., Mrsulja, B. J., Spatz, M. and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. V. Ultrastructural changes in H3 sector of the hippocampus. Acta Neuropath. 36: 285-294, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02176-03 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Action of cerebral ischemia on decreased levels of catechol and indol amine metabolites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Mrsulja	Professor Biochem.	Inst. Biochem., Belgrade
OTHER:	B. J. Mrsulja	Research Physician	Inst. Biol.,Belgrade
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

Institutes of Biology and Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia

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SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WOF (200 words or less - underline keywords)

This project was completed in Fiscal Year 1976. The resulting paper has since been published:

Mrsulja, B. B., Mrsulja, B. J., Spatz, M. and Klatzo, I.: Catecholamines in brain ischemia - effects of α-methyl-p-tyrosine and pargyline. Brain Res. 104: 373-378, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02192-02 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Dynamic profiles of the ischemic brain edema

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Fujimoto	Visiting Scientist	Univ. W. Ontario
OTHER:	I. Klatzo	Chief, Neuropath. Neuroanat. Sci.	LNNS NINCDS
	R. M. Graham	Biologist	LNNS NINCDS
	J. T. Walker, Jr.	Biol. Lab. Tech.	LNNS NINCDS

COOPERATING UNITS (if any)

University of Western Ontario, London, Ontario, Canada

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.4	PROFESSIONAL: .5	OTHER: .9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using the specific gravity determinations on abnormal uptake of water by the brain tissue was followed in gerbils which were subjected to cerebral ischemia by occlusion of the common carotid artery at the neck. The significant edematous change was demonstrable in various brain structures subjected to ischemia within 10 minutes following occlusion. In the post-ischemic periods, animals which developed necrotic changes revealed extremely low specific gravity of the affected tissue and a conspicuous damage of the blood-brain barrier, as demonstrated by the extravasation of the Evans blue tracer. This project has been completed.

Project Description:

Objectives: Edema constitutes the most important complication of cerebral ischemia. Therefore, an evaluation of the mechanisms involved in ischemic brain edema is of great clinical relevance. Objective of this study is to apply a sensitive technique for demonstration of an abnormal water uptake by the brain tissue and to correlate the obtained data with the concommitant behavior of the blood-brain barrier and histological tissue changes. This should elucidate the nature of ischemic brain edema.

Methods Employed: Mongolian gerbils are subjected to unilateral occlusion of the common carotid artery at the neck for various periods of time and sacrificed either with the remaining clip or at various time intervals following release of the occlusion. For evaluation of the blood-brain barrier (BBB) changes, the animals are injected with 2% solution of Evans blue. Following the sacrifice by decapitation, the brains are rapidly removed and submerged in kerosene. Small samples representing the cerebral cortex, hippocampus and basal ganglia are removed from the ischemic and the opposite control hemispheres. Visible blue discoloration of the brain due to abnormal penetration of the Evans blue into the brain tissue is recorded and photographed. The small samples are dropped into the gradient column of kerosene and bromobenzene prepared according to the method of Nelson (Nelson, S. R., Manz, M.-L. and Maxwell, J. A.: Use of specific gravity in the management of cerebral edema. J. Appl. Physiol. 30: 268-271, 1971). The specific gravity of the samples is recorded using standards. The corresponding brain tissue is fixed by immersion in buffered paraformaldehyde and the histological slides are prepared and stained with Cresyl Violet and H & E methods.

Major Findings: An abnormal water uptake expressed by lowering of specific gravity of the brain tissue samples was demonstrable already after 10 minutes of unreleased ischemic occlusion. The lowering of specific gravity progressed with the duration of the occlusion up to 6 hours after which it remained unchanged when estimated at 9 hr occlusion. When the occlusion was released after 1 hr ischemia, the amount of edema during the first 5 post-ischemic hours remained unchanged. During the 5 to 20 hour post-ischemic periods, two groups of animals could be recognized: (1) in one group the abnormal water uptake remained unchanged, and (2) the second group of gerbils showed extremely low specific gravity of the tissue on the side of occlusion. In these animals it was possible to correlate the low specific gravity values with the damage of the BBB and the histological picture of severe tissue necrosis.

Significance to Biomedical Research and the Program of the Institute: The study on ischemic brain edema is of great clinical relevance. It is obvious that an ischemic brain injury is characterized by marked heterogenicity concerning the intensity of changes, ranging from irreversibly damaged tissue to that showing only relatively slight changes. It can be assumed that by controlling the complicating edema large territories of brain tissue affected by ischemia can be recovered and the final extent of ischemic tissue distinction can be greatly reduced. Application of some rational and effective measures for control and treatment of an ischemic insult can be possible by precise elucidation of the mechanisms involved in the development of the ischemic brain edema.

Proposed Course of the Project: This project has been completed and the findings were presented at the International Workshop on Dynamic Aspects of Cerebral Edema, Montreal, Canada.

Publications:

Fujimoto, T., Walker, J. T., Jr., Spatz, M. and Klatzo, I.: Pathophysiologic aspects of ischemic edema. In Pappius, H. M. and Feindel, W. (Eds.): Dynamics of Brain Edema. Berlin Heidelberg, Springer Verlag, 1976, pp. 171-180.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02281-01 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Pathophysiologic correlations in the disturbed cerebrovascular permeability due to air embolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Nishimoto	Visiting Fellow	LNNS NINCDS
OTHER:	M. Wolman	Visiting Scientist	LNNS NINCDS
	J. T. Walker, Jr.	Biol. Lab. Tech.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.1	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Air embolism produced in the brains of Mongolian gerbils served as experimental model to correlate various parameters of the increased cerebrovascular permeability. 0.03-0.05 ml of air injected into the internal carotid artery resulted within a few minutes in a conspicuous breakdown of the BBB to the Evans blue albumin tracer. An increased permeability to protein tracers could also be shown by the application of horseradish peroxidase and it was demonstrable for approximately 5 hours. On the other hand, an abnormal passage of smaller molecule tracers such as sodium fluorescein and C^{14} sucrose was apparent even 24 hours following air embolism. Correlation of the BBB changes with cerebral blood flow (CBF) assessed by C^{14} antipyrine radioautography revealed an increased CBF in early stages and areas of decreased CBF in later stages associated with edema. Radioautography to demonstrate the utilization of C^{14} deoxyglucose (Kennedy's technique) demonstrated areas of greatly increased glucose phosphorylation to glucose-6-phosphate, without any spatial relationship to areas with abnormal BBB to various tracers.

Project Description:

Objectives: The main objective of this study has been to interrelate various changes which follow abnormal cerebrovascular permeability produced by a brief interruption of blood supply due to air emboli. Specifically it was intended to elucidate a possible relationship of BBB disturbances to changes in the CBF and to their metabolic effect on the brain parenchyma, as expressed by glucose utilization radioautographic assay.

Methods Employed: Mongolian gerbils were subjected to injection of 0.03-0.05 ml of air into the external carotid artery through a catheter introduced near the bifurcation of the common carotid artery, the injected air travelling with the blood via the internal carotid artery into the brain.

For study of the abnormal permeability of the BBB, 2% Evans blue solution, 10% sodium fluorescein and C^{14} sucrose solution were used. The changes in water content were measured by specific gravity determinations of the tissue samples. The CBF was radioautographically assayed after administration of C^{14} antipyrine one minute before the sacrifice. The glucose utilization was studied radioautographically sacrificing the animals 45 minutes following injection of C^{14} 2-deoxy-D-glucose. Frozen sections were prepared and photographed for the localization of fluorescent (Evans blue and sodium fluorescein) BBB tracers. The same sections were subjected to radioautography to demonstrate the distribution of C^{14} antipyrine and glucose analogue.

Major Findings: The injection of air produced damage of the BBB demonstrable within a few minutes with protein tracers. The peak in intensity of Evans blue extravasation was 30-45 minutes following embolization and the abnormal passage of protein tracers could not be demonstrated after 5 hours. On the other hand, the abnormal passage of sodium fluorescein and C^{14} sucrose was conspicuous even 24 hours after air injection. The CBF assessment with C^{14} antipyrine revealed areas completely deprived of CBF when tested at 10 minutes following air injection. Such areas (presumably due to presence of air) were not present in brains of gerbils sacrificed 20 or 40 min later. These animals showed areas of an increased blood flow, mostly in the basal ganglia. The CBF radioautography 24 hours after embolization revealed mostly in the white matter areas of lesser grain density, presumably as the result of edematous changes. At this time no spatial relationship between CBF changes and sodium fluorescein extravasation was evident.

The radioautography following C^{14} 2-deoxy-D-glucose administration showed in the early post-embolic stages areas of intense density in the cortex, hippocampus and basal ganglia. Such areas were frequently present in the hemisphere contralateral to air injection and with no apparent spatial relationship to areas of abnormal passage of the BBB tracers. In the later stages the radioautography showed very dark, "streaky" and small circular regions, suggesting their perivascular localization.

Significance to Biomedical Research and Program of the Institute: The homeostatic regulation of the optimal biochemical environment by the BBB is severely disturbed in a number of pathological conditions. It is possible that an abnormally increased permeability of cerebral vessels can adversely influence metabolism and function of brain parenchyma. This study is focused on elucidation of interrelationships between various changes related to abnormal vascular permeability in order to recognize operative pathomechanisms in the development of resulting brain tissue injury. It can be assumed that an understanding of sequences in pathogenic events can provide an opportunity to modify these changes and influence the extent of resulting brain lesions.

Proposed Course of the Project: This study will be continued to acquire more data and it will be extended to include electron microscopic and electrophysiologic observations.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02282-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of cisternal injection of Ringer solution combined with intraperitoneal injection of glucose on survival rate of Mongolian gerbils subjected to bilateral occlusion of carotid arteries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Smialek	Visiting Scientist	LNNS NINCDS
OTHER:	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS
	J. T. Walker, Jr.	Biol. Lab. Tech.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Injection of 0.02 ml of Ringer solution into cisterna magna of Mongolian gerbils was administered 2 hours before subjecting the animals to bilateral occlusion of the common carotid arteries for 15 minutes. Immediately before the ischemic occlusion the gerbils were given 2 ml of 5% glucose intraperitoneally. Mortality of these animals following the release of clipping was compared with that in other groups consisting of: a) gerbils subjected to bilateral carotid occlusion alone, b) gerbils subjected to occlusion and administration of glucose and c) gerbils which received intracisternally Ringer solution 2 hours before the occlusion, but not glucose. The group of gerbils subjected to Ringer solution and glucose injections prior to occlusion showed 80% survival rate after 30 days of clip release. This compared with 40% survival of animals subjected to injection of Ringer solution before occlusion. The survival rate of gerbils subjected to occlusion alone or occlusion with intraperitoneal glucose injection was 20% and 15% respectively. Assays of the main energy metabolites revealed no significant differences between various groups.

Project Description:

Objectives: The main rationale for this study is a search for measures which would significantly influence the clinical course and the outcome of an ischemic brain injury. In a serendipitous way a procedure has been discovered which dramatically reduces the mortality rate (from 80% to 20%) of Mongolian gerbils subjected to bilateral occlusion of the common carotid arteries for 15 minutes. The most important objective in this study remains the elucidation of factors responsible for such improvement in survival of animals exposed to a severe cerebral ischemia.

Methods Employed: The following groups of animals were used in this investigation: 1) sham operated animals, 2) animals subjected to bilateral clipping of the common carotid arteries of the neck and followed for 30 days after the release of occlusion, 3) animals which at the time of occlusion received 2 ml of 5% glucose intraperitoneally, 4) animals which were injected with 0.02 ml of Ringer solution 2 hours prior to carotid occlusion, and 5) gerbils in which bilateral carotid occlusion was combined with intracisternal Ringer solution and intraperitoneal glucose injections at the times specified in the previous groups. Mortality rate in all groups was determined during 30 days. Independently, similar groups of animals were sacrificed by submersion in liquid nitrogen: 1) immediately following release of occlusion, 2) 4 hours, 3) 24 hours, and 4) 30 days after release of the clips. The brains of these animals were dissected and the blocks of tissue from the cerebral cortex, hippocampus and thalamus were assayed for brain glucose, brain glycogen, brain ATP, phosphocreatine, lactate and pyruvate. Glucose was assayed also in blood samples.

Major Findings: The survival rate of different groups of animals were as follows: a) bilateral, 15 minute carotid occlusion alone: approx. 20%; b) carotid occlusion plus glucose injection: approx. 15%; c) intracisternal Ringer solution injection prior to carotid occlusion: approx. 40%; and d) intracisternal Ringer plus intraperitoneal glucose injection in bilaterally occluded gerbils: above 80%! Assays for blood and brain glucose, brain glycogen, brain ATP, phosphocreatine, lactate and pyruvate showed no significant differences between various groups.

Significance to Biomedical Research and Program of the Institute: An effort to influence the clinical course of animlas subjected to cerebral ischemia has been of great importance, since the experimental findings could lead to improvements in the clinical management of stroke patients. A dramatic difference in the survival rate (80% v. 20%) of animals which, in addition to being subjected to bilateral ischemic occlusion, received intraperitoneal glucose and intracisternal Ringer solution injections warrants further studies on the pathomechanisms involved. Also, it will be of importance to reproduce a similar effect in a different species and in a different experimental model of ischemia.

Proposed Course of the Project: Further investigations on this project will be concentrated on the elucidation of mechanisms which account for the remarkable difference in survival. Biochemical assays in various groups of animals will be extended to include biogenic amines, transport phenomena and dynamics of the CSF. The effect of Ringer and glucose solutions will be investigated in several other models of cerebral ischemia.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01995-05 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Morphological studies of CNS demyelination and remyelination in model systems.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. deF. Webster	Associate Chief	LNNS NINCDS
OTHER:	T. Tabira	Visiting Fellow	LNNS NINCDS
	M. J. Cullen	Staff Fellow	LNNS NINCDS
	N. Sternberger	Research Associate	LNNS NINCDS
	B. Trapp	Guest Worker	LNNS NINCDS
	S. H. Wray	Assoc. Prof. Neurology	Harvard Medical School
	F. Wolfgram	Prof. Neurology	UCLA Medical School
	M. W. Kies	Chief, Section on Myelin Chemistry	LCM NIMH

COOPERATING UNITS (if any)
Department of Neurology, Johns Hopkins Medical School, Baltimore, Maryland
Department of Neurology, Harvard Medical School, Boston, Massachusetts
Department of Neurology, UCLA Medical School, Los Angeles, California

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cellular Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.3	4.0	2.3

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to use morphological methods such as light and electron microscopy, freeze-fracture techniques, electron dense tracers, and immunochemical staining to study the lamellar structure of CNS myelin and mechanisms of myelin formation and breakdown. In vivo models of a CNS myelinated tract, the optic nerves of Xenopus tadpoles and the myelinated epiretinal fibers of the rabbit eye are used and current studies include myelinotoxic effect of cerebrospinal fluid from multiple sclerosis patients, methods to stain basic protein in myelin and oligodendrocytes and the appearance of myelin lesions produced by cycloheximide and hexachlorophene.

Project Description:

Objectives: 1) Using the tadpole optic nerve system developed in this laboratory to test additional human cerebrospinal fluid (CSF) samples, to correlate myelinotoxicity with clinical and other laboratory data, and to begin investigating the mechanism of the myelinotoxic effect. 2) To use ethanolic phosphotungstic acid (E-PTA) and immunocytochemical methods to study the distribution of basic proteins (BP) in CNS myelin and oligodendrocytes. 3) To compare the electron microscopic appearance of lesions produced in myelinating optic nerves of tadpoles by cycloheximide with those found after maintaining tadpoles at low temperature. Also, to test whether hexachlorophene (HCP) lesions in myelinated fibers of the rabbit retina could be well visualized by examining retinal whole mounts with the differential-interference microscope and finally, to study their electron microscopic appearance.

Methods Employed: 1) Groups of *Xenopus* tadpoles were injected with samples of coded CSF or a saline control solution; 2 days later, optic nerve whole mounts were made, myelin lesions were counted, analyzed statistically, decoded and correlated with patients' clinical and laboratory data. To investigate the mechanism of the myelinotoxic effect, fractions of several positive CSFs were prepared and retested. 2) To study junctional complexes and other specializations in myelin forming cells, optic nerves of *Xenopus* tadpoles or myelin fractions were stained *en bloc* with E-PTA or with rabbit antibovine BP according to the peroxidase-antiperoxidase method. 3) Groups of stage 52-55 tadpoles received a 5 µl injection of 1.5×10^{-3} - 7.1×10^{-6}M cycloheximide or were immersed in a 7.1×10^{-5}M solution. After 12-72 hrs., the tadpoles were sacrificed and their optic nerves were prepared for electron microscopic studies. In experiments utilizing rabbit retinas as a model of a myelinated tract, the vitreous overlying the retina of 20 day and 6 wk old rabbits was injected with 0.1 ml of HCP (20 µg/ml) or a saline control solution. At intervals thereafter, the eyes were enucleated, hemisected, the vitreous teased free, and the posterior poles were immersed in fixative. After 2 hrs., the retina was gently separated from each posterior pole; pairs of comparable areas were selected so that the microscopic appearance of the set used to prepare whole mounts could be compared with those processed for phase and electron microscopic study.

Major Findings: 1) Our *in vivo* test has now been used to test the myelinotoxicity of 88 CSF samples. Two thirds of the 33 CSFs that were obtained from patients with a clinical diagnosis of definite MS had myelinotoxic activity which correlated best with the patients' clinical state at the time of lumbar puncture. If done less than 3 wks after the onset of an acute attack or progressive symptoms, and if the patient had received no steroid therapy, more than 90% of the CSFs were positive. Only 33% were myelinotoxic if obtained from MS patients on steroid therapy or from those who were stable clinically or who had progressive disease for more than 3 wks. Significant ($P < .05$) myelinotoxic activity was found in 50% of CSFs from patients with possible MS, 30% of CSFs from patients with optic neuritis and 22% of CSFs from patients hospitalized for other neurological diseases. When fractions of a few positive CSFs were tested, several IgG fractions were positive. Additional tests are needed to substantiate these preliminary results. 2) in sections of tadpole optic nerves, there was a moderately dense layer of E-PTA staining beneath inner membrane leaflets of oligodendrocyte tongue processes that extended to compact myelin's major dense line. Intensely stained microtubules were found in oligodendrocyte perikarya, tongue processes and terminal loops. Post embedding treatment of Xenopus frog optic nerves with sera containing antibodies to bovine, chick and frog BP produced staining that was restricted mainly to the inner (axonal) and outer (extracellular) margins of intact myelin sheaths. In sections of isolated myelin, multi-layered whorls with characteristic myelin periodicity were heavily stained. 3) In sections of nerves from tadpoles exposed to cycloheximide, oligodendrocytes were severely altered. The number of polyribosomes in their perikarya was greatly reduced and the granular endoplasmic reticulum was disorganized. Many oligodendrocyte tongue processes at inner margins of myelin sheaths were enlarged, were filled with vesicular profiles and occasionally indented axons. Focal changes in the lamellar structure of myelin were found at paranodal regions. In flat, unstained whole mount preparations of rabbit retinas, normal myelin sheaths or those containing vacuoles typically seen in HCP intoxication were readily visualized over long distances and appeared as if longitudinally sectioned when examined with the differential interference microscope. In sections prepared for electron microscopic study, intramyelinic junctional complexes were found at margins of the HCP lesions.

Significance to Biomedical Research and the Program of the Institute: 1) Results of testing 88 CSF samples have shown that CSF from MS patients is myelinotoxic if obtained from untreated patients during an acute attack or in clinical progression. There is no correlation of the myelinotoxic effect with CSF cell count, total protein, IgG or IgM levels. These results suggest that active demyelination in acute MS produces a myelinotoxic substance (perhaps an IgG) that often can be found in CSF. More test results are needed to identify the factor, confirm the correlation with the clinical state of the MS patient and determine whether CSF from patients with other types of CNS myelin breakdown is also myelinotoxic. 2) Under the conditions used in our experiments, E-PTA stains basic proteins. The distribution described seems to correspond to precursors or fragments of myelin BP since it was not changed by incubation with DNase, RAase, neuroaminidase or hyaluronidase. Also, staining was abolished by acetylation treatment, was absent after trypsin digestion, and its distribution was similar to that seen after immunostaining with anti-BP antibodies. The E-PTA staining pattern during rapid myelination suggests that BP is synthesized in the granular endoplasmic reticulum, transported by microtubules and incorporated into oligodendrocyte membranes that surround tongue processes and terminal loops. Results from immunocytochemical experiments now in progress will provide additional evidence needed to interpret the E-PTA findings. 3) These observations and SDS gel studies of optic nerve proteins show that cycloheximide and low temperature alter oligodendrocytes' ability to synthesize and insert membrane components containing protein into CNS myelin. Similar lesions could not be produced by slowing axonal transport, inhibiting cholesterol biosynthesis or by acute injury (axonal transection). The whole mount technique, becuase of its relative simplicity, increases the usefulness of the rabbit retina model for studying mechanisms of CNS demyelination.

Proposed Course of the Project: To be continued. The above findings have been presented at annual meetings of the Society for Neuroscience, the American Society for Neurochemistry, and the American Association of Neuropathologists.

Publications:

Tabira, Y., Webster, H. deF. and Wray, S. H.: Multiple sclerosis cerebrospinal fluid produces myelin lesions in tadpole optic nerves. New Eng. J. Med. 295: 644-649, 1976.

Tabira, T., Webster, H. deF. and Wray, S. H.: In vivo test for myelinotoxicity of cerebrospinal fluid. Brain Res. 120: 103-112, 1977.

Cullen, M. J. and Webster, H. deF.: The effects of low temperature on myelin formation in optic nerves of Xenopus tadpoles. Tissue & Cell 9: 1-10, 1977.

Brodsky, F. M., Wray, S. H., Tabira, T. and Webster, H. deF.: Preparation of whole mounts of rabbit retina for examination with the differential-interference microscope: A technique for morphological studies of CNS demyelination. Brain Res. 124: 140-146, 1977.

Webster, H. deF., Tabira, T. and Reier, P. J.: Examination of the developing nervous system of Xenopus tadpoles with differential-interference microscopy: A new assay procedure for neurotoxicologists. In: Roizin, L., et al. (Eds.): Neurotoxicology. New York, Raven Press, 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01996-05 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Membrane structure in CNS tissue and subcellular brain fractions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. deF. Webster	Associate Chief	LNNS NINCDS
OTHER:	R. H. Quarles	Research Chemist	DMN NINCDS
	R. McIntyre	Visiting Fellow	DMN NINCDS
	P. J. Reier	Asst. Prof. Anat.	Univ. of Maryland
	J. M. Matthieu	Asst. Prof. Pediatrics	Univ. of Lausanne

COOPERATING UNITS (if any)
Developmental and Metabolic Neurology Branch, NINCDS
Department of Anatomy, University of Maryland School of Medicine, Baltimore, Md.
University of Lausanne School of Medicine, Lausanne, Switzerland

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cellular Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.5	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to use freeze fracture techniques and electron microscopy to study the structure of myelin and cell membranes in CNS tissue and in subcellular fractions. Current experiments include the examination of myelin related fractions (R. McIntyre, R. Quarles), and axolemma enriched fraction (J. M. Matthieu) and hexachlorophene intoxication in freeze-fracture replicas (P. Reier).

Project Description:

Objections: 1) To study electronmicroscopically myelin related fractions discarded during the myelin isolation procedure of Norton and Poduslo (1973). 2) To study the fine structure of myelin and axolemma-enriched fractions isolated from rabbit brains. 3) To use electron dense tracers and freeze-fracture replicas to investigate the pathogenesis of hexachlorophene (HCP) induced CNS myelin lesions.

Methods Employed: 1) Brains and spinal cords of adult rats were used to prepare myelin and myelin-related fractions. Portions of these fractions were fixed, dehydrated, embedded and sectioned for electron microscopic examination. 2) Brains of adult rabbits were used to prepared myelin and axolemma-enriched fractions which were processed and sectioned for electron microscopic examination. 3) Groups of stage 54-58 Xenopus tadpoles were immersed in 0.2 μg/ml. HCP solution. At intervals thereafter, 10 μl of horseradish peroxidase (HRP) were injected around the right optic nerve of several tadpoles before processing their nerves for electron microscopic study. At each interval other tadpoles were fixed by perfusion. Their optic nerves were gently isolated, glycerinated and used to prepare freeze-fracture replicas which were studied with the electron microscope.

Major Findings: 1) A fraction which floated on .32M sucrose (F) and the material released on osmotic shocking of the myelin fraction with water (W) were studied electron microscopically. Fraction F consisted of fairly large single membrane profiles and some membrane whorls with the periodicity of myelin. However the whorls in F were smaller than those seen in isolated myelin. Fraction W contained small single membrane vesicles and some small myelin fragments. 2) The axolemma-enriched fraction was collected at the 1.0/1.2 interface of the sucrose gradient. In electron micrographs, there were many single membrane profiles that varied in size. Contaminants included some mitochondrial fragments and a few whorls of compact myelin. 2) At all intervals studied, the tracer, HRP, was distributed throughout the optic nerve parenchyma. However, none was observed within the intramyelinic vacuoles indicating that HCP did not significantly alter the permeability of tight junctions at outer or inner tongue processes of oligodendrocytes or those between adjacent paranodal loops. In electron micrographs of freeze-fracture replicas, these tight junctions were normal in appearance. However, in membrane regions around myelin vacuoles there were particle free elevations corresponding to multilamellar blebs seen projecting into the vacuoles in thin sections.

Significance to Biomedical Research and the Program of the Institute: 1) Both the biochemical and electron microscopic data obtained for the W fractions suggest that they contain myelin related membranes such as parts of the oligodendroglial plasmalemma that surround tongue processes and lateral loops in the paranodal region. The F fraction, which also contains vesicles may represent myelin fragments dissociated during isolation. Since fractions similar to F in composition and electron microscopic appearance have been isolated from diseased white matter, further study of this fraction may provide a better understanding of demyelinating processes.

2) The chemical and electron microscopic data show that the 1.0/1.2 fraction is enriched in axolemma, it differs dramatically from purified myelin and does not resemble previously studied heavy myelin subfractions or myelin-related fractions. Though contaminants of mitochondrial and myelin origin were found, this fraction should be useful in studying metabolic relationships of axons to myelin and oligodendroglia. 3) The presence of particle free areas on blebs bordering intramyelinic vacuoles may reflect focal alterations of myelin membrane structure and composition that are associated with HCP intoxication. The normal appearance of tight junctions at tongue processes and paranodal loops in freeze-fracture replicas as well as their impermeability to macromolecules such as HRP is additional evidence that these tight junctions do not have a primary role in the development of the HCP lesion. However, they may be important in preventing blister rupture and breakdown of the vacuolated myelin internodes.

Proposed Course of the Project: To be continued. The above findings have been presented at Annual Meetings of the American Association of Anatomists, the American Association for Neurochemistry, and the Society for Neuroscience.

Publications:

McIntyre, R. J., Quarles, R. H., Webster, H. deF. and Brady, R. O.: Isolation and characterization of myelin-related membranes. J. Neurochem. 1977 (in press).

Matthieu, J.-M., Webster, H. deF., Beny, J. and Dolivo, M.: Characterization of myelin and axolemma-enriched fractions from rabbit brain. Brain Res. Bull. 1977 (in press).

Reier, B. J., Tabira, T. and Webster, H. deF.: Hexachlorophene-induced myelin lesions in the amphibian central nervous system: A freeze-fracture study. J. Neurol. Sci. 1977 (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01442-11 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Permeability of Cellular Layers in the Vertebrate Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. S. Reese	Head, Sect. on Functional Neuroanat.	LNNS NINCDS
OTHER:	R. L. Gulley	Senior Staff Fellow	LNO NINCDS
	D. M. D. Landis	Dept. Neurology	Mass. Gen. Hosp.
	D. M. McDonald	Cardiovascular Research Inst.	UCSF
	G. L. Rasmussen	Guest Worker	LNNS NINCDS

COOPERATING UNITS (if any)

Laboratory of Neuro-Otolaryngology, NINCDS
Cardiovascular Research Institute, University of California, San Francisco

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Functional Neuroanatomy

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.05	OTHER: 0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The location and nature of extracellular barriers to colloidal substances at a variety of locations in the nervous system has been determined. In the organ of Corti, extensive systems of tight junctions form continuous bands surrounding cells and occluding extracellular spaces. It is these bands of junctions which control the movement of colloidal and probably ionic substances in intervening extracellular spaces. No tight junctions are found at the astrocytic border of the brain but other membrane specializations revealed by the freeze-fracture technique suggest that these cells also participate in the blood-brain barrier system. Furthermore, these membrane structures disappear rapidly in anoxia, suggesting that they depend on metabolic factors for their maintenance. These data on the cellular basis of brain barriers, as well as the new structural approaches used in these studies, afford a basis to design experimental studies of a variety of clinically important conditions which depend on or produce damage to brain barriers. They also give rise to new ideas about the roles of various cells associated with neurons and about the function of barrier systems in special regions, such as the organ of Corti.

Project Description:

Objectives: Determination of the structural basis of biologically and clinically important barriers in the central nervous system, peripheral nervous system, and in sense organs.

Methods Employed: a) The organ of Corti in chinchillas was examined with the freeze-fracture technique to determine the types and patterns of junctions between the various types of epithelial cells that separate the endolymph from perilymph. The permeability of these junctions to proteins was tested by injecting horseradish peroxidase into the subarachnoid space, from where it diffused into the perilymph, and then using the electron microscope to determine the distribution of peroxidase in the organ of Corti. b) The astrocytic sheaths at the arachnoid surface of the brain and at the interfaces of the brain with blood vessels in mice were examined with the freeze-fracture technique in brain slices incubated in appropriate medium bubbled with O_2 or N_2.

Major Findings: a) Barriers in the organ of Corti of the mammalian inner ear were the object of a collaborative study with Dr. Robert Gulley. The perilymph is separated from the endolymph by tight junctions between the apices of hair and supporting cells. These tight junctions prevent peroxidase injected into the perilymph from reaching the endolymph.

The freeze-fracture technique was used to examine the architecture of these junctions in more detail. They are more extensive, and presumably, "tighter" than in any other tissue studied so far. The unique form of these tight junctions also suggests that they might have functions other than to prevent mixing of endolymph and perilymph and, perhaps, are involved in the first steps in the sensing of mechanical displacements in the organ of Corti.

In the course of studying the tight junctions other nonjunctional specializations of the lateral borders of the hair cells were noted. In fact, these specializations, which are not found on any other types of cell, clearly distinguish the inner from the outer hair cells.

b) The freeze-fracture technique was used to study the structure of astrocytic membranes, particularly where they surround cerebral blood vessels, or form the outermost surface of the brain next to the cerebrospinal fluid. These membranes are characterized by many assemblies of small particles that are never found on neurons and are less frequent elsewhere on astrocytic membranes. These assemblies were examined in slices of cerebellum incubated in artificial cerebral spinal fluid for up to 30 minutes. They were maintained if the medium was bubbled with O_2 but disappeared in N_2. They also disappeared from the intact brain if the circulation was interrupted for 15 minutes or more.

Significance to Biomedical Research and the Program of the Institute: We determined the location of barriers to proteins at significant fluid interfaces in the brain and sense organs. Our determinations depend on cytological techniques that show specifically which components of which layers are permeable. Thus, we are able to determine the cells as well as the probable mechanisms that control the chemical environment of the brain and related structures. This year we defined, for the first time, the cellular and subcellular basis of the barrier between the endolymph and perilymph. These techniques are now available for clinically oriented research to see the role of pathological changes in these barriers in various diseases of the middle ear. Also of interest is that our recent data indicate a possible role for astrocytes in the brain barrier system. This possibility will focus attention on the role of these glial cells in pathological conditions affecting the brain barrier system.

Proposed Course of the Project: The final papers on the organ of Corti have been published or are in press. No further work on this subject is planned. The work on the effects of oxygen lack on the assemblies of small particles in astrocytic membranes was repeated in the last year and we plan to prepare a paper on this subject. The next step is to find a line of cultured astrocytes whose plasma membranes produce assemblies in vitro, so that the assemblies can be isolated and characterized. However, we do not have the personnel to be able to spend time on this project in the next year.

Publications: Gulley, R.L. and Reese, T.S.: Intercellular junctions in the reticular lamina of the organ of Corti. J. Neurocytol. 5: 479-507, 1976.

Gulley, R.L. and Reese, T.S.: Freeze-fracture studies of the non-junctional membrane specializations in the organ of Corti. Anat. Rec., 1977 (in press).

McDonald, D.M. and Rasmussen, G.L.: An ultrastructural analysis of neurites in the basal lamina of capillaries in the chinchilla cochlear nucleus. J. Comp. Neurol. 173: 475-495, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01881-07 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Structural Basis of Synaptic Transmission

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. S. Reese	Head, Sect. on Functional Neuroanat.	LNNS NINCDS
OTHER:	K. Lynch	Biologist	LNNS NINCDS
	R. L. Ornberg	IPA Biologist	LNNS NINCDS
	D. W. Pumplin	IPA Biologist	LNNS NINCDS
	R. P. Rees	Guest Worker	LNNS NINCDS
	S. Cohen	Sr. Staff Fellow	BBB NICHD
	M. B. Rheuben	IPA Biologist	LNNS NINCDS
	M. Dubois-Dalcq	Chief, Section on EM	IDB NINCDS
	C. Franzini-Armstrong	Dept. Biology	Univ. of Penna.
	R. L. Gulley	Sr. Staff Fellow	LNO NINCDS
	M. Henkart	Sr. Staff Fellow	BBB NICHD
	J. E. Heuser	Dept. Physiology	UCSF
	D. M. D. Landis	Dept. Neurology	Mass. Gen. Hosp.

COOPERATING UNITS (if any)
Behavioral Biology Branch, NICHD
Infectious Diseases Branch, NINCDS
University of Pennsylvania, Philadelphia, Pennsylvania

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Functional Neuroanatomy

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.3	3.9	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to determine the location and mechanism of neurotransmitter secretion and reception. Rapid freezing and subsequent freeze-fracture of synapses visualizes fleeting structural changes in the cell membrane accompanying discharge of synaptic vesicles. This approach has shown that each quantum of transmitter is released by one synaptic vesicle. Structural details which may be specific for different pharmacological types of synapses are being investigated by examining postsynaptic membrane structure. New methods are being tried for marking specific entities, such as receptors, to identify them in freeze-fracture and thin section preparations. This work is significant in that it defines the normal structure of synapses and relates normal variations in structure to different functional states. Thus, it becomes possible to distinguish pathological changes in structure, an issue of great importance in studying the etiology of epilepsy or myasthenia gravis. The current program also includes freeze-fracture of developing synapses, which will aid in understanding of both normal development and developmental failures in the brain and peripheral nervous system.

Professional Personnel Engaged on the Project (cont.):

S. Nakijima	Dept. Biology	Purdue Univ.
E. Raviola	Dept. Anatomy	Harvard Med. Sch.
B. J. McLaughlin	Dept. Anatomy	Univ. Tennessee

Cooperating Units (cont.):

Laboratory of Neuro-Otolaryngology, NINCDS
UCSF Medical School, San Francisco, California
Massachusetts General Hospital, Boston, Massachusetts
Purdue University, West Lafayette, Indiana
Harvard Medical School, Boston, Massachusetts
University of Tennessee, Memphis, Tennessee

Project Description:

Objectives: Synapses are sites where electrical signals pass between neurons or between neurons and muscle cells. This project seeks to find the exact location and mechanism of synaptic transmission in the central and peripheral nervous systems in both adult and immature animals, and in tissue cultured neurons and muscles.

Methods Employed: Synapses are prepared for examination in the electron microscope by the freeze-fracture technique in order to visualize the internal structure of their surface membranes. The vertebrate nerve-muscle synapse has been the main object of study in the last year. In addition, nerve-muscle synapses from insects have been examined because they use glutamate rather than acetylcholine as a neurotransmitter, and the giant synapse from the squid has been examined because its physiological condition can be defined so precisely. Isolated muscles are exposed to a variety of different ionic environments in conjunction with different schedules of electrical nerve stimulation, black widow spider venom, and botulinum toxin. They are then put in an aldehyde fixative, or frozen in a special apparatus designed in our laboratory, in order to capture fleeting structural changes associated with synaptic vesicle discharge and recovery. They are then freeze-fractured and the resulting replicas of split membranes examined in a high resolution electron microscope. Alternatively, they are freeze substituted for examination in thin sections.

For studies of developing synapses, developing cerebellum or retina is fixed and freeze-fractured by conventional methods. For studies of synapse formation in culture, cultured neurons are fixed and stained with special methods to display the nature of the initial contacts which lead to synapse formation. Alternatively, the development of postsynaptic receptors in cultured muscle cells is followed by freeze-fracturing areas of acetylcholine sensitivity previously identified with fluorescent bungarotoxin. A special method had to be developed in our laboratory to freeze-fracture identified regions of cultured muscle fibers.

Major Findings: This year the section published a method for capturing fleeting structural changes in functioning synapses by freezing them very rapidly, thereby avoiding the use of chemical fixatives and cryoprotective agents which alter profoundly the structure of synapses. By freeze-fracturing rapid-frozen neuromuscular synapses, it has been possible to see, and count, synaptic vesicles fusing with the synaptic plasmalemma of synaptic terminals at several different levels of transmitter secretion. It turns out that each quantal secretory event results from the fusion of one synaptic vesicle with the plasmalemma. Since the temporal resolution of rapid freezing in the machine used by this section is less than 2 msec, as measured by a capacitance method developed here this year, the section could hope to follow the fate of synaptic vesicle membrane after vesicles fused with the synaptic plasmalemma. In less than 0.1 sec, the vesicle membrane is completely flattened out into the plasmalemma. Components of the vesicle membrane, appearing as particles after freeze-fracturing, then spread out randomly, finally to be collected a second later in little particle islands which are parts of the coated vesicle system. The final fate of these components of the vesicle membrane is reincorporation into synaptic vesicles. This finding of particle recycling extends earlier work of the section showing that local recycling of synaptic vesicles replaces those lost during synaptic activity.

Parallel studies of other types of synapses were made using either rapid-freezing or conventional fixation to prepare them for freeze-fracturing. The giant synapse in the squid was freeze-fractured for the first time and it was shown that this synapse had well defined synaptic vesicle release areas. Thus, it can be used for studies of synaptic activity in which the state of the synaptic terminal at the time of fixation can be defined precisely with microelectrodes. Synapses in the insect which use glutamate as a transmitter show patterns of synaptic vesicle release and recovery similar to those at the frog neuromuscular junction.

This year a study was completed which applied the freeze-fracture technique to neuromuscular synapses to see why such medically important biological substances as botulinum toxin and black widow spider venom have such profound effects on synapses. Results indicate that the toxin specifically blocks exocytosis and that the venom lets calcium ions in to excite exocytosis. One of the most interesting findings, which supports these conclusions, is that the venom can partially overcome the effect of the toxin, so that the normal distribution of exocytosis reappears. The clinical usefulness of this finding is limited however, unless a way can be found to return the exocytosis to control by nerve impulses arriving at the synapse.

The extent of local recycling was measured by stimulating isolated synapses for up to 48 hours and then looking for depletion of synaptic vesicles or surface membrane. So far, no depletion of membrane has been found, even in preparations where axoplasmic stores of membrane were excluded, either by blocking axoplasmic transport with colchicine or by ligating the nerve near the muscle.

The success of the rapid freezing and freeze substitution techniques in producing realistic views of labile membrane structures has led to exploration of several nonsynaptic systems in order to explore the uses of this new technique. In the toad retina, changes in spacing of the photoreceptor membranes in light and dark were resolved. Changes in the sarcoplasmic reticulum of muscle during contraction coupling were also seen.

The freeze-fracture technique has also yielded new information about the structure of the postsynaptic membrane. Particulate structures, thought to be receptor molecules within the postsynaptic membrane, appear to be different at each chemical type of synapse. This year, the squid giant synapse, the insect neuromuscular junction, synapses in the mammalian inner ear and synapses at the termination of auditory fibers in the brain stem were examined. It is of interest that the postsynaptic membrane at the squid giant synapse, the insect neuromuscular junction, the auditory terminals in the brain stem, and the sensory terminals at inner hair cells resembled each other but differed from both central nervous system inhibitory terminals in the cerebellum and olfactory bulb, and from excitatory cholinergic terminals in muscle and ganglia. While the transmitter in the squid synapse and mammalian inner ear are not known, the transmitters at the other two examples of this type of synaptic junction are thought to be amino acids. It is also of interest that the postsynaptic membrane at the inhibitory terminals in the middle ear, which are thought to be cholinergic, resembles that at central nervous system inhibitory synapses rather than at excitatory cholinergic synapses.

Several other current studies with the freeze-fracture technique have begun to yield new data on changes in the structure of synaptic membranes during development. At the developing presynaptic membrane, studied in the chick retina, components of the presynaptic specialization appear first in small islands which subsequently fuse to form the large adult specialization. In developing chick muscle in culture, particle aggregates of the type associated with receptors in the adult appear at spots of acetylcholine sensitivity (inferred from binding of fluorescent bungarotoxin) prior to the arrival of the nerve terminals. The changes in synaptic membranes which accompany initial contact between nerve terminals and target cells were studied in cultures of sympathetic ganglia, using a technique for staining cell coats in thin sections. This revealed a new form of junction which forms and then disappears as synaptic junctions form, suggesting that these initial contact junctions could have a role in the induction of synapse formation. However, careful study with the freeze-fracture technique of similar junctions at sites of future synapses in the developing cerebellum failed to reveal any special structures inside membranes at these initial contact junctions. Thus, it is possible that these structures are confined to the surface coat of developing synaptic membranes.

Also a mutant mouse, weaver, was studied because in this cerebellar mutant, the parallel fibers degenerate before synapses are formed. Nevertheless, normal postsynaptic particle aggregates were found on spines which had never contacted a parallel fiber. In this system, at least, the development of the postsynaptic specialization does not appear to depend on intimate contact with a presynaptic terminal.

A high resolution scanning electron microscope was acquired in the last year and this instrument was used to reveal the structural organization of surfaces of the postsynaptic membrane at greater levels of detail than has previously been possible. However, this technique is still too new to be certain yet of the significance of the new surface features of the postsynaptic or other membranes which it reveals. Thus, the studies so far with this instrument have been chiefly directed towards development of techniques to take advantage of its high resolution. Work on viral development has continued because the simplicity of these preparations makes them good subjects with which to develop and extend techniques to examine the cell membrane.

Finally, freeze substitution is being performed on squid axons to look for rapid changes in structure subsequent to nerve impulses.

Significance to Biomedical Research and the Program of the Institute: One of the most immediately practical aspects of the studies on synapses is that they define the normal structure of various types of synapses in a variety of functional states. This knowledge will permit distinction between normal and pathological, as well as between resting and active synapses, with the electron microscope. In structural studies of epileptic brains, it should now be possible to distinguish normally active from pathologically active synapses. Similarly, in diseases involving peripheral nerve-muscle synapses at neuromuscular junctions, it may well be possible to distinguish pathological states from changes resulting from increased or decreased activity. The finding that different chemical types of synapses are distinguishable by the freeze-fracture technique may contribute to the task of determining the chemical organization of synapses in the central nervous system. Knowledge about the locations and pharmacological types of various central nervous system synapses will make it possible to understand the action of drugs on the brain on a cellular level. Our new studies on the development of synapses may reveal reasons why development or repair of synaptic systems is sometimes unsuccessful.

Our program of developing and adapting the freeze-fracture technique to study neural structure has been helpful to other program areas of NINCDS, as evidenced by the fact that major programs in neuroviruses, otolaryngology, and multiple sclerosis have found it important to make, with our assistance, major commitments to setting up facilities to perform research with this technique. In every instance, their primary investigators were trained in this technique in the Section on Functional Neuroanatomy.

Proposed Course of the Project: Much of the work outlined above is currently being prepared for publication, or has been submitted. The major work on rapid changes in frog neuromuscular synapses will be finished and manuscripts submitted next year.

A major new direction is to extend the rapid freezing and freeze substitution techniques to new areas of synaptic and membrane physiology. A second direction is to use the scanning electron microscope and the freeze-fracture technique to study developing synapses. How are areas of pharmacological sensitivity formed? What interactions between the pre- and post-synaptic processes control their formation?

Publications: Gulley, R.L. and Reese, T.S.: Freeze-fracture studies of synapses on the organ of Corti. J. Comp. Neurol. 171: 517-544, 1977.

Dubois-Dalcq, M., Reese, T.S. Murphy, M. and Fuccillo, D.: Defective bud formation in human cells chronically infected with SSPE virus. J. Virology 19: 579-593, 1976.

Dubois-Dalcq, M., Reese, T.S. and Narayan, O.: Membrane changes associated with assembly of visna virus. Virology 74: 520-530, 1976.

Dubois-Dalcq, M., Rodriguez, M., Reese, T.S., Gibbs, Jr., C.C. and Gajdusek, D.C.: Search for a specific marker in the neural membranes of scrapie mice (a freeze-fracture study). Lab Invest. 36: 547-563, 1977.

Pumplin, D.W. and Reese T.S.: Action of brown widow spider venom and botulinum toxin on the frog neuromuscular junction examined with the freeze-fracture technique. J. Physiol., 1977 (in press).

Heuser, J.E., Reese, T.S. and Landis, D.M.D.: Preservation of synaptic structure by rapid freezing. Cold Spring Harbor Symposia on Quantitative Biology 15: 17-24, 1976.

Landis, D.M.D. and Reese, T.S.: Structure of the Purkinje cell membrane in staggerer and weaver mutant mice. J. Comp. Neurol. 171: 247-260, 1977.

Henkart, M. Landis, D.M.D. and Reese, T.S.: Similarity of junctions between plasma membranes and endoplasmic reticulum in muscle and neurons. J. Cell Biol. 70: 338-347, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01805-09 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Membrane Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Anders	IPA Biologist	LNNS NINCDS
	D. Schmechel	Research Associate	LCS NIMH
OTHER:	M. W. Brightman	Head, Section on Neurocytology	LNNS NINCDS
	P. Marangos	Chief, Unit on Neurochemistry	LCS NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The freeze-fractured membranes of subpial (marginal) and perivascular astrocytes contain orthogonal arrays or assemblies of small particles as do ependymal cells. Assemblies do not occur in choroid plexus epithelium. The functions of the assemblies are unknown but might have something to do with secretion or absorption of fluid and solutes. Solutions of 10^{-4} N ouabain and of acetazolamide, which reduces CSF production, are being infused ventriculo-cisternally in mice. We are also attempting to change the number or configuration of the assemblies by causing marginal astrocytes and ependyma to proliferate or hypertrophy after painting 30% to 66% gels of aluminum hydroxide onto the floor of the IV ventricle and the dorsal surface of the medulla. In order to determine whether such assemblies are present in astrocytes and ependyma cultured in vitro we must first identify the cells as glial or fibroblastic. To make this distinction, a cytoimmunochemical technique is used whereby fixed cells in situ and in vitro, are labeled with an antibody to a "non-neuronal protein" (NNE) which, we have found, labels astrocytes, ependyma and choroid epithelium but not neurons.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To vary the number or configuration of intramembranous assemblies by different treatments in order to determine the role of these protein particles.

Methods Employed: The marginal glia and the ependyma of intact rodents and those given, intracisternally, ouabain, acetazolamide and aluminum hydroxide are freeze-cleaved. Organ cultures of brain are to be examined with a cytoimmunochemical technique, which we have found distinguishes neurons from glia *in situ*, in order to be certain of the identity of cell clusters *in vitro*. Clusters of "undifferentiated" glial cells will then be cleaved in order to see whether they too have assemblies.

Major Findings: Neurons and astrocytes *in situ* can be distinguished by means of a peroxidase conjugated antibody to a nerve specific enolase (NSP) and to a liver enolase present in astrocytes, ependyma and choroidal epithelium (NNE). The NSP is not found in glia or epithelium and the NNE is not found in neurons. Freeze-cleaved marginal astrocytes show some variation in packing and length of intramembranes assemblies in normal rodents.

Significance to Biomedical Research and the Program of the Institute: By manipulating the intramembranous particles of astrocytes we may learn something of how these cell membranes are involved in the activity of normal glial cells and, eventuallv, of developing and neoplastic astrocytes.

Proposed Course of the Project: To examine the membranes of cultured normal astrocytes, of reactive astrocytes within injured brain, and of astrocytoma cells.

Publications: Prescott, L. and Brightman, M.W.: The sarcolemma of *Aplysia* smooth muscle in freeze-fracture preparations. *Tissue and Cell* 8: 241-258, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02086-04 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regeneration in Vertebrate and Invertebrate Nerves

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Rosenstein	Guest Worker	LNNS NINCDS
OTHER:	M. W. Brightman	Head, Sect. on Neurocytology	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pieces of the superior cervical ganglion (SCG) transplanted into a cerebral ventricle can survive. Prior to transplantation, pieces of SCG from the recipient or from a donor, are labeled with carbon black which was quickly incorporated by presumed capsule cells and macrophages. The fragments of SCG, examined 7 days to 90 days after implantation, contain ganglion cells which, by light microscopy, appear normal. Peripheral to the ganglion cells are carbon-labeled capsule cells. Not only are SCG neurons, that had been isolated from their pre- and postsynaptic connections, easily recognizable but the extent of the implant itself appears, upon gross inspection, as a readily discerned ink-speckled mass on the floor of the IV ventricle. Many vessels containing red blood cells lie within the explant and it thus appears that the transposed tissue has become revascularized. Surprisingly, the implant becomes covered by choroid plexus epithelium and by ependyma. The stroma of the implant intermingles with that of the choroid plexus and eventually becomes confluent with the subependymal neuropil of the medulla.

Project Description:

Objectives: To determine whether isolated pieces of peripheral and central nervous system (CNS) can grow within cerebrospinal fluid (CSF) compartments and to induce the regenerating axons to grow into selected regions of the CNS across intact ependyma and glia.

Methods Employed: Portions of SCG from donor rats that are 21 days old or more are immersed briefly in a solution of biologically inert carbon black and then inserted gently upon the floor of the IV ventricle beneath the cerebellar nodulus of the same rat (autograft) or of other recipient rats from 7 to 14 days of age (heterograft). Other implants are placed over the pia of the medulla so as to overlie glia instead of ependyma and choroid plexus. Care is taken not to injure either glia or epithelium. After various periods, the recipient is fixed with aldehydes and osmium for light and electron microscopy.

Major Findings: Implants from newborn donors degenerate while implants from older rats, particularly those older than 21 days, survive. The surviving implants become enclosed by choroidal epithelium and ependyma and the stroma of the implants appears to become continuous with the parenchyma of the brain. The isolated neurons in the implant appear normal at the light microscopic level and often have elongated processes extending from their somata. Blood vessels, situated mainly at the periphery, supply the transplanted SCG fragment.

Significance to Biomedical Research and the Program of the Institute: This *in situ* "culture" of neurons should enable scanning electron microscopy and freeze-fracture observations of growing axons before and after they make contact with epithelium, glia and neurons. This system might serve well to see how and where neurites and blood vessels regenerate and how they may be directed to make contact with specific neurons.

Proposed Course of the Project: This project was initiated 5 months ago. We shall see if and where autonomic axons grow across intact ependyma and marginal glia. By deafferenting the external cuneate nucleus which lies very near the pial surface, we shall see whether SCG axons can grow across an intact external limiting membrane, invade the underlying nucleus, and take the place of the absent dorsal root terminals.

Publications: Shivers, R.R. and Brightman, M.W.: Trans-glial channels in ventral nerve roots of crayfish. J. Comp. Neurol. 167: 1-26, 1976.

Shivers, R.R. and Brightman, M.W.: Formation of hemi-desmosomes during regeneration of crayfish nerve root sheath as studied with freeze-fracture. J. Comp. Neurol. 173: 1-22, 1977

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02144-03 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Hypertension on the Permeability of Cerebral Endothelium to Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Brightman	Head, Section on Neurocytology	LNNS NINCDS
OTHER:	M. Sato	Visiting Fellow	LNNS NINCDS
	J. S. Robinson	Neurosurgery Resident	Univ. Chicago
	S. I. Rapoport	Neurophysiologist	LNP NIMH

COOPERATING UNITS (if any)
Department of Neurosurgery, University of Chicago, Chicago, Illinois
Laboratory of Neurophysiology, NIMH

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Is there a common mechanism by which protein movement across capillaries is enhanced after a number of unrelated stimuli? We have reinvestigated the effects of hyperosmotic solutions on the blood-brain barrier (BBB) to horseradish peroxidase (HRP). We had previously found that the intracarotid infusion of 2.0 M urea solutions resulted in the penetration of HRP between tight junctions in cerebral endothelium. When 1.4 M arabinose is so infused in rats, the great majority of interjunctional clefts are not penetrated yet HRP is exuded from vessels. A second possible mechanism, vesicular transport, may be responsible for the exudation. We have eliminated this possibility by the following control experiment: The hyperosmotic solution is infused, the brain is fixed by aldehydes and then from 1 to 3 hours post-fixation, the HRP is infused into the aorta. Fixation eliminates vesicular transfer yet the pattern of exudates is very similar to that which developed in living animals and are confined to the side receiving the arabinose. The conclusion is that the HRP may have crossed via actively formed channels that remain patent long enough to be fixed in the open position or by actual injury and wide separation of a few endothelial cells.

Project No. Z01 NS 02144-03 LNNS

Project Description:

Objectives: To determine the way in which protein crosses blood vessels in which the blood-brain barrier (BBB) has been lowered.

Methods Employed: The intravenous injection of aramine or a bolus of saline to produce transient hypertension, or the intracarotid infusion of hyperosmotic 1.4 M arabinose are used to open the BBB to peroxidase (HRP). In order to determine the role of vesicular transport, some rats were infused with arabinose, fixed, and then perfused with HRP long after vesicular transport would have been halter by the fixation.

Major Findings: In both hypertensive and hyperosmotic conditions HRP rapidly exudes from cerebral vessels. In rats given hyperosmotic arabinose, very few is any endothelial tight junctions are penetrated by the HRP. There is an increase in the number of HRP labeled vesicles in some capillaries. It is unlikely, however, that only the vesicles transfer the protein because exudates appear around vessels in rats fixed before HRP was infused.

Significance to Biomedical Research and the Program of the Institute: The reversible, non-destructive opening of the BBB will enable the administration of therapeutic agents and replacement enzymes that are normally inaccessible to the brain. Conversely, by understanding the mechanism of protein and peptide transfer, a rational approach may be devised to prevent the opening of the BBB so as to prevent the entry of unwanted substances.

Proposed Course of the Project: To determine the roles of channels, formed actively or as a result of injury, and of vesicles in the transcapillary passage of protein.

Publications: Brightman, M.W. and Robinson, J.S.: Some Attempts to Open the Blood-Brain Barrier to Protein. In McLaurin, R.L. (Ed.): *Head Injuries*. New York, Grune & Stratton, 1976, pp. 107-113.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02145-03 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Identification of Neurons having Terminals in the Median Eminence and Area Postrema

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. D. Broadwell	Staff Fellow	LNNS NINCDS
OTHER:	M. W. Brightman	Head, Sect. on Neurocytology	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The conditions necessary for retrograde transport of blood-borne peroxidase having been established, those required for orthograde transport within intact, undisturbed neurons are being investigated. Neuronal cell bodies and dendrites are exposed to horseradish peroxidase (HRP) by perfusing it through the cerebral ventricles. Neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) are labeled by this route. A very small amount of HRP moves down the axons of these neurosecretory cells to accumulate with a few lysosomes in their axonal terminals located in the posterior lobe of the pituitary gland. The ingestion of a 1.5% NaCl solution, known to enhance the anterograde flow of neurosecretory peptides (Gainer et al., 1977), also increases the amount of HRP that flows down the neurosecretory axons to the posterior lobe. However, the HRP remains within the cerebral extracellular fluid for long periods in the dehydrated mice. Thus, the increased amount of HRP in their axons may simply be due to a decreased turnover of CSF. Neuronal somata and dendrites are consequently bathed by HRP for a sufficiently long period for more protein to be incorporated and transferred to their axons.

Project Description:

Objectives: To follow the orthograde as well as the retrograde transport of blood-borne protein in various neurons.

Methods Employed: Peroxidase (HRP) is perfused ventriculo-cisternally for 10 minutes in normal mice and in those given 2 per cent sodium chloride solution to drink for 5 days. The mice were fixed from 6 to 24 hours later.

Major Findings: An appreciable amount of HRP is incorporated by the dendrites and somata of cranial motor neurons and of neurosecretory cells. The amount is never as great, however, as that incorporated by their axonal arborizations. The protein is transported in small amounts from neurosecretory somata to their axon terminals in the median eminence and neurohypophysis. Considerably more HRP appears in the axons and their terminals of dehydrated mice, because the HRP is cleared by bulk flow from pericellular spaces much more slowly in these mice. Thus, somata and dendrites can take up more protein to be transferred orthogradely. Smooth endoplasmic reticulum and vacuoles are involved in both retrograde and anterograde axonal transport.

Significance to Biomedical Research and the Program of the Institute: The conditions under which neurons can transport protein and peptides both orthogradely and retrogradely provide an understanding of how some toxic or therapeutic substances may be handled by these cells. Reducing the turnover of CSF may also enable exceedingly small amounts of HRP to be used in determining the source of axon tracts and, thereby, minimize damage and diffusion that can obfuscate such results.

Proposed Course of the Project: To follow the orthograde route in ligated cranial motor axons and in regenerating neurosecretory axons.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02200-02 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Freeze-Fracture of Cell Membranes Intercalated with Lipids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Sato	Visiting Fellow	LNNS NINCDS
OTHER:	M. W. Brightman	Head, Section on Neurocytology	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The distribution of fatty acid (FA) in cells of blood vessels and brain is readily visible by both light and electron microscopy when 0.1 to 0.3 ml of linolenic acid are infused intravascularly in very high concentrations for ready detection, over a period of 10 minutes. At the end of this time, the vessels and brain are fixed either by aldehydes and osmium or, by osmium solutions only. Not only are FA droplets randomly distributed throughout endothelial and smooth muscle cells, but in some vessel segments, most of the droplets are within pinocytotic pits and vesicles. Normally, FA circulates within the blood in combination with serum albumin and the droplets within pits that take up protein may represent the FA-albumin complex. Within the cerebral parenchyma, the synaptic vesicles of a few axon terminals contain FA droplets and the postsynaptic web in the dendrites of some areas, are heavily stained by the linolenate.

Project No. Z01 NS 02200-02 LNNS

Project Description:

Objectives: To determine the distribution of fatty acid (FA) visible by both light and electron optics, within blood vessels and neurons.

Methods Employed: High concentrations of linolenic acid, which is strongly osmiophilic, is infused into one carotid artery or through the cerebral ventricles of rats and the tissue prepared for electron microscopy and freeze-fracture.

Major Findings: FA droplets, probably in the form of sodium and potassium soap, occur not only randomly across cell membranes and in the cytoplasm of endothelial cells but, in segments of the basilar and carotid arteries, the great majority of droplets fill pinocytotic vesicles. Within the cerebral parenchyma, some synaptic vesicles are also labeled by the FA and the post-synaptic web of dendrites are heavily stained.

Significance to Biomedical Research and the Program of the Institute: FA is normally carried in the serum in combination with albumin. The droplets may represent such a fraction and has provided a glimpse as to how some of the FA can enter cells physiologically. There appears to be some component of synaptic vesciles and webs which combine with FA. This staining may provide some information as to the physical nature of these structures.

Proposed Course of the Project: Over fifty rats have been infused and the results are sufficiently consistent to warrant publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01449-11 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The effect of hormones on the retrograde neuronal reaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Cammermeyer	Head, Section on Experimental Neuropathology	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NIH, NINCDS, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rabbits subjected to facial nerve transection during cortisone acetate treatment display acute depression of regenerative changes in neuronal perikarya and of reactive mitosis in microglial cells. To determine whether these reactions can result in a more severe impairment of neuronal regeneration, chronic experiments have been started for quantitative studies. A comparison with thyronine-treated animals is carried out in order to establish the nature of the action of the two hormones on the central nervous system. This may have some relevance to the prognostication of clinical observations and treatment.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine what effect hormonal treatment may have on the recovery phases of motor neurons subjected to transection of their axons.

Methods Employed: Rabbits were treated with cortisone or thyronine for a week prior to severance of the facial nerve.

Horseradish peroxidase powder was applied to the cut end in order to assess the intensity of axonal flow in cortisone treated and non-cortisone treated animals. The peroxidase technique was used also for determining the area which is occupied by neurons undergoing retrograde reaction.

After short and prolonged survival, the animals were sacrificed and fixed by perfusion in deep anesthesia.

Serial sections of the brain stem were stained for glycogen and with PAS-gallocyanin.

Major Findings: Preliminary studies revealed that, after cortisone treatment, there is in the operated animals a depression of reactive mitosis in microglial cells and an acute depletion of glycogen in neurons. The acute neuronal reaction is the same after thyronine treatment as in untreated animals. Preliminary review of a pilot study with horseradish peroxidase indicates that axonal flow is not intensified after cortisone treatment of operated young animals.

The horseradish peroxidase technique revealed (1) the site of perikarya sending axons to various facial muscles differs from that indicated in other studies, and (2) the area with peroxidase tagged neurons is smaller than that occupied by neurons undergoing acute retrograde reaction as demonstrated by conventional staining methods.

After experimental operations designed to study the chronic effect of these two hormones, no differences in clinical manifestations have been detected.

Significance to Biomedical Research and the Program of the Institute: After a study on the acute depressing effect of cortisone on neuronal perikarya and neuroglial elements, it is of interest to determine whether an opposite reaction can be obtained by introduction of another hormone, such as thyronine, which is said to accelerate peripheral nerve regeneration. By couting the number of cells remaining in the facial nucleus after single operation or reoperation, one should be in a position to conclude decisively whether administration of either of the two hormones can be of any benefit or can affect the vulnerability of nervous tissue. Definite guidelines for clinical application must await results of such experiments.

Proposed Course of the Project: (1) preparation and examination of histologic material from hormone-treated animals with acute and prolonged survival after single transection or reoperation. (2) To test the effect of cortisone on the axonal flow with horseradish peroxidase in animals in which the facial nerve was transected at varying ages. (3) To improve the fixation procedure in order to optimize the histochemical staining technique.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02002-05 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mast cells in the brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cammermeyer	Head, Section on Experimental Neuropathology	LNNS NINCDS
OTHER:	W. Flor	Research Scientist	AFRI

COOPERATING UNITS (if any)

Department of Neurobiology, Armes Forces Radiobiology Institute,
Bethesda, Maryland

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The functional role of mast cells, which are diffusely dispersed in the brain and aggregate in larger numbers in paraventricular regions, is still disputed. These cells are known to contain several biochemically active substances enabling them to prevent coagulation, control blood circulation, and take an active part in transmission of nerve impulses and in neurovegetative functions. On the basis of morphologic characteristics, the mast cells are considered to be in a labile stage of activity which can progress to exhaustion and cell death. By a quantitative study of such cell forms, indirect evidence of interaction between these cells and nervous tissue elements may be established. To this end, electron microscopic and quantitative studies have been initiated.

Project Description:

Objectives: To determine the significance of cytoplasmic pallor and nuclear pyknosis of mast cells in the central nervous system in an effort to clarify the functional role of these cells.

Methods Employed: Examination of microscopic sections from the brains of various animals with the aid of a specific staining technique for mast cells.

Major Findings: The appearance of mast cells varies greatly; pallor, as an expression of loss of biologically active material, is often associated with loss of the nucleus.

Significance to Biomedical Research and the Program of the Institute: Since mast cells contain a variety of substances - heparin, histamine, serotonin, dopamine, etc. - which are known to act on coagulability and viscosity of blood, diameter and permeability of blood vessels and neuronal function, and since these cells occur in many parts of the brain but in varying numbers, it is assumed that their presence depends on functional requirements. If this is the case, they may play a role in controlling blood flow and neuronal interaction in the brain. The microscopic demonstration of pallor and nuclear loss may suggest that mast cells disintegrate *in situ* as their functional capacity is exhausted.

Proposed Course of Project: The specificity of degranulation and nuclear pyknosis in mast cells is to be investigated in serial sections of brain stem in different animal species, some of which have been treated with compound 48/80. To compare the mast cells in various parts of the brain with those in peripheral nerves.

In another investigation, started in collaboration with Dr. William Flor, AFRI, the electron microscopic characteristics of mast cells in different functional stages will be ascertained.

Publications:

Cammermeyer, J.: Factors contributing to denucleation of cerebral mast cells. Acta Anat. 96: 459-468, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02003-05 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The thalamo-choroidal body.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cammermeyer	Head, Section on Exper. Neuropath.	LNNS NINCDS
OTHER:	E. Westergaard	Lektor	Univ. of Copenhagen

COOPERATING UNITS (if any)
Anatomy Department C, University of Copenhagen, Copenhagen Ø, Denmark

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The taenia thalami in the cat has a complex structure for which the term thalamo-choroidal body has been coined. A study of its submicroscopic structure is contemplated in order to help to clarify its functional role, which, as yet unknown, is conjectured to be the control of blood flow to the choroid plexus of the third ventricle.

Project Description:

Objectives: To subject the taenia thalami in cat to an ultramicroscopic survey because of its prominence in this species.

Methods Employed: The variety of elements in this region have been identified by conventional light microscopic techniques.

Major Findings: A region rich in blood vessels and collagenous fibers, nerve cells, supporting glial elements, plasma cells and mast cells occupies the taenia thalami of the cat.

Significance to Biomedical Research and the Program of the Institute: On the basis of its complex structure, it has been speculated that this region may serve a chemoreceptive or baroreceptive function.

Proposed Course of the Project: To examine the thalamo-choroidal body in electron micrographs.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02004-05 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The pathogenesis of myelopathies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Cammermeyer	Head, Section on Exper. Neuropath.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Terminated.

ONIAN SCIENCE INFORMATION EXCHANGE T NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02143-03 LNNS

OD COVERED
J ly 1, 1976 to September 30, 1977

E OF PROJECT (80 characters or less)
lumetric changes of brains during histologic preparation

ES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER ESSIONAL PERSONNEL ENGAGED ON THE PROJECT

I: J. Cammermeyer Head, Section on Exper. Neuropath. LNNS NINCDS

COOPERATING UNITS (if any)

None

/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

For the purpose of assessing changes in volume of the brain during fixation, a special device has been designed and a baseline of volumes for different species has been established.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To measure the volume of brains from small animals in order to test the effect of fixation and dehydration.

Methods Employed: An apparatus was designed for direct measurement of small brains utilizing the same principle as laid down earlier in the design of an apparatus for volumetric measurement of segments of the intact spinal cord. Simultaneous measurement of the brain weight permitted calculation of the specific gravity.

Conventional histologic techniques are employed.

Major Findings: When brains of animals of different species were fixed by perfusion first with saline and then with Bouin's solution, their specific gravity remained constant.

The volume of the Bouin-fixed brains was unaffected by flow, rate, amount or temperature of the fixative.

Solitary dark neurons were found in zones of transition between gray and white matter.

Significance: For standardization of the histologic preparation of the brain, it is important that volume of tissues be controlled. Constancy in volume reflects adequacy of the preparatory technique, a requirement for estimating the status of the neurons. The results obtained may provide a baseline for assessing the effect of experimentally produced damage or edema in the brain.

Proposed Course of Project: To compare the changes in volume of brains during the process of fixation by immersion and by perfusion. To formulate recommendations for overcoming inadequacies in methods of preservation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02193-02 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Fat emboli in normal brains fixed by perfusion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cammermeyer	Head, Section on Exp. Neuropath.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In control animals fixed by perfusion, isolated fat emboli occur in scattered cerebral blood vessels. Their origin from connective tissue fat released in the course of autopsy is supported by (1) the histologic demonstration of fat on the surface of the brain, (2) the occurrence of fat emboli in pial arteries and in superficial cerebral blood vessels which have been torn, (3) the greater frequency of fat emboli when the brain is covered with oil, and (4) the prevention of such emboli when the brain is exposed under water. They are associated with post-mortem removal of the brain and do not reflect an intravital circulation of fat. In acute fat embolism induced by injection of oil, the emboli are separated by closely packed erythrocytes; under such conditions, a transport during life becomes evident. This project is completed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To assess the morphologic characteristics, source and mode of development of fat emboli in normal animals fixed by perfusion.

Methods Employed: Animals were fixed by perfusion, and oil red O fat-stained frozen sections were prepared from representative blocks of the brains.

Major Findings: In approximately 25% of the normal rabbits and cats fixed by perfusion, a few isolated fat emboli occur in veins and arteries near the cerebral surface. Their formation was found to depend on accumulation of tissue fat on the brain surface during the autopsy, tearing of the leptomeninges and superficial cerebral blood vessels, and suction caused by stretching and constriction of blood vessels during removal of the brain. When the brain is exposed under continuous flow of water, the development of such false emboli can be prevented.

For comparison, oil was injected into blood vessels and the animals were sacrificed shortly thereafter. Such fat emboli in perfused-fixed material abut columns of closely packed erythrocytes. After prolonged intervals between injection and perfusion, tissue changes of different types develop. The absence of any such changes in the normal perfused-fixed animals indicates that the isolated emboli in these animals had not circulated during life.

Significance to Biomedical Research and the Program of the Institute: The observation that fat emboli occur in perfused-fixed normal material invalidates the diagnostic value of such vascular occlusions unless the autopsy was performed under special precautions by the application of running water. The occurrence of single or isolated fat emboli in empty vascular channels can not be taken as a sign of intravital circulation in material fixed by perfusion; even in material fixed by immersion, similar precautions during removal of the brain should be followed in order to assure unequivocal diagnosis. The simultaneous occurrence of fat and erythrocytes is an important criterion of intravital transport of fat.

Proposed Course of the Project: This project is completed.

Publications:

Cammermeyer, J.: Cerebral and retinal fat emboli in normal animals fixed by perfusion. Acta Anat. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02194-02 LNNS

PERIOD COVERED
July 1, 1967 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Fat emboli in normal flattened retina fixed by perfusion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cammermeyer	Head, Section on Exp. Neuropath.	LNNS NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The unexpected demonstration of fat emboli in flattened retina of perfused-fixed animals is associated with influx of extraneous fat during removal of the eye and preparation of the flattened retina. Embolization of this type can be prevented by ligating the optic nerve prior to removal of the eye. This project is completed.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 02194-02 LNNS

Project Description:

Objectives: To determine the frequency, origin and diagnostic criteria of fat emboli in the retina of animals fixed by perfusion.

Methods Employed: In animals fixed by perfusion, the optic nerve was ligated and the retina carefully separated from the sclera. The loosened retina, after staining with oil red O, was flattened on a slide and covered with glycerin gelatin.

Major Findings: On rare occasions, fat emboli were demonstrable in the flattened retina of cats which had been fixed by perfusion. The emboli occluded larger blood vessels but did not usually enter into the smallest blood vessels or capillaries. The blood vessels were free of erythrocytes. After injection of oil, the fat emboli occluding numerous blood vessels formed a wide-meshed network of embolized capillaries. In order to prevent formation of the emboli, the optic nerve was ligated prior to removal of the eye and during flattening of the retina. The corpus vitreum contained large amounts of fat, which should be flushed away with running water.

Significance to Biomedical Research and the Program of the Institute: Since fat emboli in normal retina fixed by perfusion can be avoided by ligation of the optic nerve, as well as by ample flushing with running water, they are regarded as artifactual in nature. The realization that such emboli may complicate experimental material is important for a correct interpretation of morphologic observations and for the unequivocal diagnosis of retinal fat embolism.

Proposed Course of the Project: Completed.

Publications: See Project No. Z01 02193-02 LNNS

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02195-02 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Perivascular cerebral siderosis as a marker of blood-brain barrier damage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the course of a study on fat embolism in the cat, a focal diffusion of iron was demonstrated ubiquitously through the brain at sites of damaged blood-brain barrier. The role of changes in serum iron concentration in the diffusion of iron is being explored in experiments on different animal species. Under such conditions, it should be possible to determine whether iron has any toxic effect on tissue elements.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 02195-02 LNNS

Project Description:

Objectives: To determine the source of focal deposition of iron.

Methods Employed: Cerebral fat embolism is induced by intracardial injection of oil. Serum iron levels are determined by the Technicon Auto-Analyzer method. Histologic material consists of paraffin sections stained by the Turnbull blue method for iron and other methods for study of different elements.

Major Findings: In animals injected with oil into the systemic circulation, there are, ubiquitously through the brain, multiple foci with iron deposited in astrocytes, microglial cells and oligodendrocytes. These foci are more numerous than the lesions observed in PAS-gallocyanin-stained sections in which a loss of nerve cells and nerve fibers, progressive changes of astrocytes and proliferative changes of microglial cells are demonstrable.

Significance to Biomedical Research and the Program of the Institute: In animals, as well as in man, because of the normal content of serum iron in the blood, the diffusion of iron may act as an inherent marker of blood-brain barrier damage. The demonstration of such damage can then be determined under conditions when it is inadvisable to inject acid semicolloidal dyes, generally used in experiments to reveal any vascular damage. In addition, the toxic effect of perivascularly deposited iron on tissue elements can be estimated and factors contributing to ferruginization of neuronal and glial elements can be explored.

Proposed Course of the Project: To examine histologically material from laboratory animals subjected to serum iron analysis following injection of oil, in order to determine any relationship between severity of serum iron changes and intensity of iron deposition in the tissue. To study iron deposition in cerebral stab wound and in electron micrographs.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02283-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Experimental cerebral embolism induced by intraperitoneal injection of oil

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cammermeyer	Head, Section on Exp. Neuropath.	LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Unlike the large infarctions formed after intravenous or intracardial injection of oil, minute cerebral lesions with iron accumulation and pericapillary hemorrhages were demonstrable throughout the brain of cats subjected to intraperitoneal injection of oil and terminal fixation by perfusion. This project is completed.

Project Description:

Objectives: To devise a technique for the purpose of inducing with oil emboli cerebral changes which will be similar to those developed after cerebral fat embolism.

Methods Employed: Oil was injected intraperitoneally in cats. The experiments were terminated by perfusion first with saline and then with Bouin's solution. Frozen sections stained with fat soluble dyes were prepared for the demonstration of oil emboli. Other methods were used for identification of tissue changes.

Major Findings: Minute lesions with loss of neuronal elements, reactive neuroglial changes and deposition of iron were produced ubiquitously in the brain. Myriads of pericapillary hemorrhages were associated with lesions.

Significance to Biomedical Research and the Program of the Institute: Injection of oil intraperitoneally was found to be more advantageous than the intravenous or intracardial routes because (1) the oil is easily introduced, and (2) the lesions are minute and scattered throughout the brain. The presence of iron in the lesions may be associated with disintegration of erythrocytes or with abnormalities in iron metabolism, the role of which is the subject of a future project (Project No. Z01 NS 02195-02 LNNS). The demonstration of pericapillary effusions of blood in material fixed by perfusion opens new perspectives in current research on the mechanism and role of cerebral hemorrhages (Project No. Z01 NS 02286-01 LNNS).

The intraperitoneal route for the development of cerebral oil embolism proved to be impractical for systematic studies since only two cats of ten cats and one dog manifested cerebral fat embolism in microscopic sections.

Proposed Course of the Project: This project is completed.

Publications:

Cammermeyer, J.: Cerebral fat embolism with focal siderosis and pericapillary hemorrhages after intraperitoneal injection of oil in cats terminally fixed by perfusion. Exp. Neurol. 55: 694-708, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02284-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Improvement of current methods of fixation by perfusion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.1	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In an effort to explain the unusual occurrence of solitary dark neurons, a material prepared under different conditions of perfusion has been reviewed. In a further attempt to improve fixation by perfusion, the adequacy of the fixation is also tested by the histochemical glycogen technique, the purpose being to obtain a method which will give optimal stainability of this substance.

Project Description:

Objectives: (1) To obtain consistent glycogen reaction in neurons and astrocytes, and (2) to prevent formation of "solitary" dark neurons.

Methods Employed: (1) The neuronal glycogen reaction is tested in animals which in narcosis were subjected to artificial respiration and fixation by perfusion with solutions containing inhibitors of glycolysis. Paraffin sections treated with dimedone are stained with periodic acid Schiff.

(2) The distribution of solitary dark neurons is ascertained in paraffin sections of brains fixed by perfusion after modification of the various steps involved in this procedure. Speed of flow of the perfusates and specific gravity of the brains were determined for groups of three animals after change of a single step in the perfusion procedure.

Major Findings: (1) Glycogen in neurons varies greatly and may be absent when perfusion is delayed three to five minutes. Use of a glycogenolytic inhibitor results in a more intense staining. When paratoluene sulphonic acid is used instead of Bouin's picric acid solution, glycogen is preserved in neurons but not in astrocytes.

(2) The speed of flow and the specific gravity of the brain are influenced by changing certain steps in the perfusion procedure such as pressure and temperature without any effect, however, on the quality of neuronal fixation. Contrary to a previous statement, removal of the brain after a brief post-perfusion interval was found to be deleterious to the appearance of the neurons. The original recommendation of a 4-hour interval for material fixed by perfusion with a strongly acid solution was confirmed.

Significance to Biomedical Research and the Program of the Institute: (1) A method which may reduce the speed of glycogen depletion is needed in order to estimate correctly the involvement of this substance in neurons and astrocytes under different experimental conditions. Also it may provide a basis to determine whether by changing the content of glycogen the vulnerability of various elements can be modified.

(2) An assessment of factors contributing to formation of the unusual solitary dark neurons is required to overcome still existing controversies concerning the role of dark neurons in experimental material.

Proposed Course of the Project: (1) To apply various inhibitors during the perfusion procedure.

(2) To search for inherent factors in the technique which contribute to formation of dark neurons. Statistical analysis of flow data.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02285-01 LNNS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Cliniconeuropathologic study of brains from Guam

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cammermeyer	Head, Section on Exp. Neuropath.	LNNS NINCDS
OTHER:	D. C. Gajdusek	Chief, Lab. CNS Studies	CNSS NINCDS
	C. J. Gibbs, Jr.	Assoc. Chief, Lab. CNS Studies	CNSS NINCDS

COOPERATING UNITS (if any)
Laboratory of Central Nervous System Studies, NINCDS

LAB/BRANCH
Labatory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Brains from patients observed in Guam are being studied histologically in order to verify the clinical diagnosis.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 02285-01 LNNS

Project Description:

Objectives: To determine the nature of cerebral changes in a heterogeneous neurologic material from Guam, as part of the Chronic Disease Studies: Slow, Latent and Temperate Virus Infections of Drs. Gajdusek and Gibbs (Project Nos. Z01 NS 00969-13 CNSS; Z01 NS 00201-22 ODIR).

Methods Employed: Gross examination and routine histologic techniques.

Significance to Biomedical Research and the Program of the Institute: The material is from patients who were examined clinically over long periods of time. Verification of the clinical material is essential for genetic studies on diseases peculiar to this region.

Proposed Course of the Project: To prepare the histologic material and report the results of examinations.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02286-01 LNNS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanism of cerebral hemorrhages

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The demonstration of cerebral hemorrhages in material fixed by perfusion offers an opportunity to examine factors involved in development of hemorrhages.

Project No. Z01 NS 02286-01 LNNS

Project Description:

Objectives: To assess factors contributing to development of petechial hemorrhages and hemorrhagic infarction.

Methods Employed: Injection of fat in systemic circulation of cats. Fixation by perfusion after varying postinjection intervals. Embedding in paraffin or plastic. Histologic techniques for staining of erythrocytes and vascular walls.

Major Findings: Petechial hemorrhages and larger hemorrhagic infarctions composed of fresh erythrocytes aggregated near sites of vascular ruptures.

Significance to Biomedical Research and the Program of the Institute: An assessment of the factors contributing to hemorrhages may help to determine whether these hemorrhages occur during life or whether they can be the cause of death. Formulation of therapeutic measures as well as interpretation of hemorrhages as the cause of death will be dictated by the results of morphologic studies.

Proposed Course of the Project: In experimental animals, to: 1) change the pressure of perfusates, 2) exchange blood with saline prior to perfusion, 3) tag erythrocytes with Evans blue solution, and 4) study vasculature in thin sections. A preliminary report, entitled "Post-embolic cerebral hemorrhages in cats fixed by perfusion," was delivered at the meeting in honor of Dr. Raymond D. Adams, Massachusetts General Hospital, Boston, Massachusetts, May 19-20, 1977.

Publications: None

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Laboratory of Neural Control, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief

Introduction

The research work in the Laboratory of Neural Control (LNLC) is concentrated primarily on analyses of the central and peripheral nervous system mechanisms that are involved in the control of movement in mammals. Primary emphasis is given to those central nervous system (CNS) structures that contain the neural organizations of motor output - i.e., the spinal cord and those regions of the brain stem and cerebral cortex which project directly to the cord. Several projects deal also with the characteristics and organization of the motor units, which are the functional quantum elements of motor output, and with sensory afferent neurons that deliver input information into the spinal cord.

A broad range of technical approaches has been used in LNLC projects, ranging from what may be called "conventional" neurophysiological methods applied to anesthetized or otherwise neurologically reduced preparations, to methods of neuroanatomical pathway tracing using exogenous proteins, to studies of muscle morphology and histochemistry, and finally, to quite novel techniques, many developed within LNLC, for recording neural and mechanical data from awake, intact animals which are confortable and either free to move or moving with minimal restraint. Both cats and monkeys have been used in this research.

Many of the newer techniques for recording neural activity in intact animals have derived from the long-standing interest of LNLC staff members in the problem of developing workable neural prostheses to aid the neurologically handicapped patient. A brisk interchange of ideas and information in this area exists between present staff and former LNLC staff members now with the Fundamental Neurosciences Program of NINCDS, as well as with other groups in this country and abroad.

Present Organization

During FY 1977, LNLC has consisted of 9 professional members, including 4 permanent staff and 5 Visiting Fellows, 4 support staff members and 1 part-time laboratory aid. Two scientists from the University of Maryland School of Medicine have collaborated as part-time Guest Workers and informal collaboration has been established with some half-dozen other investigators at NIH and in other laboratories in this country and in Canada.

LNLC has never been divided into categorical "Sections", partly because of its small size but mainly because the various projects ongoing in the Laboratory are closely inter-related in goals and methods, and there is considerable interchange of staff members between them. Nevertheless, the research work of LNLC can be roughly divided on the basis of methodological approach into four categories:

1. Projects that involve more-or-less conventional neurophysiological methods, aimed at clarifying details of cellular physiology and neural circuit organization of motor systems in reduced preparations (primarily cats, either anesthetized or decerebrated under anesthesia);

2. Projects that utilize novel techniques of data acquisition from awake and intact cats and monkeys which are moving freely or moving with minimal restraint.

3. Theoretical analyses of problems of neural stimulation or of data processing which are important to the interpretation of experimental results and to the design of future experiments; and

4. Purely technical and developmental activities concerned with the generation and refinement of techniques for recording neurally-relevant data from the central and peripheral nervous systems of moving animals.

The staff of LNLC includes members with backgrounds in clinical medicine and neurology, in various aspects of physiological research, and in biomedical engineering and computer sciences. This range of expertise, together with the close-knit interaction that has characterized the group, has resulted in considerable cross-fertilization of ideas and approaches to motor system research.

Project Summaries

Almost all of the in-house work of LNLC on motor control mechanisms has to do with the cellular biology and neural organization of the spinal cord and of those supraspinal systems that project to spinal motor mechanisms. The ultimate output elements of the CNS motor system are the motor units, which are the focus of attention in a project entitled "Intrinsic Properties of Motor Units". A motor unit includes a spinal motoneuron plus the set of muscle fibers innervated by it, and in this project, methods appropriate to the study of the inter-relation between the neural and muscular elements have been devised. Building upon previous work on normal motor unit populations in the cat hindlimb pools, we have continued a long-term investigation of the effects on motor unit populations of chronic alterations in mechanical demand, specifically of the atrophic process that follows long-term immobilization of hindlimb joints, and of the effects of chronic spinal cord lesions (hemisections or complete spinal transsections). Immobilization atrophy does not produce recognizable changes in the types of motor units present in cat hindlimb muscles but does affect the different types of units present to variable extents. The most marked changes were found among the units innervating muscle fibers relatively resistant to fatigue, which are ordinarily the units used most frequently in producing postural and locomotory activities. In addition, chronic immobilization appears to produce a diminution in the strength of a reliably measured synaptic input system, that from group Ia spindle afferents to affected motoneurons, which may reflect a degree of synaptic "plasticity" responding to altered functional usage. Such studies provide useful information as to the extent to which motor unit populations can be altered by mechanical demand on the system, a problem important to the interpretation of clinical studies (usually electromyographic or muscle biopsy) in patients with altered motor function. We have continued studies of normal motor unit pools in a study of the anatomy of particular motor nuclei using retrograde transport of exogenous horseradish peroxidase (HRP), which labels both alpha and gamma motoneurons. Studies at the light microscopic level are now complete and we are beginning ultrastructural examination of this material in collaboration with outside laboratories.

Closely allied with the above is a second project entitled "Motor Control Systems in the Spinal Cord". The primary focus of this project is to elucidate how the various types of motor units present within a single motor nucleus are controlled by spinal and supraspinal motor organizations to produce precisely graded and energetically efficient force output. During the past year, we have confined attention to a poorly understood polysynaptic pathway, involving spinal segmental interneurons, which is activated by afferents from the skin of the foot and ankle and which tends to produce excitation primarily in the fast twitch motor units of ankle extensor muscles. This input organization is quite different in overall pattern from that found by us earlier for several other input systems, which excite the slow twitch motor units most effectively. Experiments in decerebrate cats suggest that this cutaneous input pathway can in fact produce preferential activation of the otherwise relatively high threshold fast twitch units under some conditions. Such differential control of different motor unit groups within the same motor pool has been the subject of past speculation but the present results have provided unambiguous evidence of its existence for the first time. Differential control systems have importance to hypotheses of motor control as well as for the interpretation of animal and human data on motor unit recruitment, a subject of both research and clinical interest. We are currently examining possible mechanisms for supraspinal control of this pathway.

The projects described above are designed to provide further insight into the functional properties and interconnections of the output elements of the motor system. However, a complete picture of the system requires information about how these elements operate during actual movements. The techniques required to provide this dynamic information from individual neural elements in moving animals have only recently become available and are still in an active state of development.

One successful approach to the problem of recording impulse traffic into the spinal cord from individual, identified primary afferent neurons is illustrated by the project entitled "Neuron Activity in Locomotion". In this work, impulses arriving in individual afferents are monitored by multiple semimicro electrodes chronically implanted into dorsal root ganglia serving the hindleg. The stability of the recording situation is such that exhaustive physiological testing is possible to identify the nature of the afferents, as well as allowing recording of their activity patterns during normal movements, including walking and running on a treadmill. The experimental design has permitted examination of a very wide range of afferent fiber types. The activity patterns in muscle spindle afferents (both primary and secondary) have given increased insight into questions about coactivation of alpha and gamma motoneurons during various types of motor behaviors, which is a subject of great current interest and debate. In addition, the results illustrate the great range of afferent input to the spinal cord that occurs during normal behavior, which must somehow be taken into account in hypotheses and conceptual models of motor control.

Part of the same project is devoted to an analysis of force production by individual hindlimb muscles during normal movement, including locomotion. The method utilizes miniature strain gauge force transducers, developed in LNLC during FY 1977, which can be implanted chronically on individual tendons. This work, although in an early stage, has already provided important information relevant to the functional usage of different types of motor units in mixed and slow twitch muscles and have raised exciting possibilities for detailed analyses of the kinesiology of the hindlimb musculoskeletal system during normal movement. The study will also utilize implanted muscle length gauges, also newly developed in LNLC. To our knowledge, the ability to monitor both muscle length and force in intact, moving animals is thusfar unique to LNLC.

A second project concerned with investigation of neuronal activity during normal movements is entitled "Cortical Mechanisms of Voluntary Motor Control." In this work, the activity of individual neurons in the motor cortex of rhesus monkeys is recorded using chronically implanted microelectrodes of a design developed and refined in LNLC over the past several years. These electrodes can record activity from the same cortical neuron, concerned with hand and arm movements, for periods of days to weeks, permitting the use of operant conditioning methods to determine precisely to which movements and muscles an individual cell is best related. Recording from groups of nearby neurons, and microstimulation of restricted sets of cortical output neurons through the same microelectrode, then permits examination of the much-debated question of whether local collections of cortical neurons (called "colonies" or "columns") are all related to the same muscle or the same movement pattern. Data collection in this type of experiment is a slow and painstaking process but it promises a degree of precision in interpretation that is considerably greater than other approaches to the question.

Several problems generated by research and by clinical situations have been dealt with in the project entitled "Models of Neural Interaction". First, it has been a consistent observation that cathodal stimulation of the cerebral cortex surface is the most effective stimulus to produce firing in the output pyramidal cells beneath the electrode. The reasons for this have been unclear and the effect is somewhat paradoxical. An analysis was undertaken of the potential profiles inside and outside of neurons with cortical cell geometry during application of surface current inputs. This model simulation study was designed to make it possible to predict what fraction of the nearby cells would be stimulated for a given current strength. The question is vital to the design of implanted visual prostheses, and is likewise important to research work on motor control using cortical stimulation as a method. This work has resulted in development of a computational model which permits such predictions, based on the available anatomical and physiological evidence, but the model now needs to be tested systematically in order to refine it. The model is "responsible" since it can, in fact, be tested experimentally and this may be possible through collaboration in the near future. A second aspect of current work in this project concerns the use of the mathematical technique of factor analysis to process data from multiple stochastic sources. Examples of such data streams abound in neurophysiology, including multi-unit neural and electromyographic recordings. Several computational advances have been made with the method in the past year which may permit practical application to data analysis of experimental signals. The next step is a critical test of the method which will use multi-unit electromyographic activity as an input.

As noted above, much of the work in LNLC depends on development and refinement of novel experimental techniques and fabrication of complex and sophisticated recording electrodes. Many of these developments have already been noted above, while others have been undertaken to benefit other laboratories at NIH, such as the fabrication of new substrates for tissue culture and a new design for micropipettes for tissue culture cells, to be used in the Behavioral Biology Branch, NICHD. Because of its importance to the program of LNLC, the project entitled "Techniques for Making Contact with the Nervous System" will be maintained as a separate and identifiable research project within the Laboratory.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01686 09 LNLC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

MOTOR CONTROL SYSTEMS IN THE SPINAL CORD

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Robert E. Burke, M.D., Chief, LNLC, NINCDS

Other Investigators: Kenro Kanda, M.D., Ph.D., Visiting Fellow, LNLC
Bruce Walmsley, Ph.D., Visiting Fellow, LNLC
John Hodgson, Ph.D., Visiting Fellow, LNLC

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bldg. 36, Rm. 5A29, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.1	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to provide information on the mechanisms operating within reflex systems which include alpha motoneurons as the output link, as well as on the interconnection and interaction of different reflex systems. Particular consideration is given to correlations between synaptic organization, intrinsic neuronal properties, dynamic behavior and the physiological characteristics of the muscle fibers innervated by the motoneurons studies.

Project Description:

Objectives: This project is designed to provide information on the organization of those neuronal systems in the spinal cord of mammals which ultimately control the discharge of alpha motoneurons, and on the interaction of primary afferent and supraspinal descending systems in the control of information flow in the segmental motor mechanisms.

Methods Employed: A variety of experimental approaches are used in this Project, but the structure of interest is the lumbosacral spinal cord of the adult cat. All work has been done on animals anesthetized with pentobartital or halothane or, in some cases, on unanesthetized decerebrate animals after destruction of the supratentorial brain under rapidly reversed gas anesthesia. Intracellular recording and stimulation methods have been used in those experiments in which the main object was to analyze synaptic potential patterns in motoneurons belonging to identified motor unit types. Most of this work in the past year has dealt with the effects of supraspinal descending fiber systems on segmental cutaneous reflex mechanisms, with supraspinal activation through stereotaxically placed electrodes. Another series of acute experiments has involved examination of the discharge patterns of soleus and medial gastrocnemius (MG) motoneurons in decerebrate animals when conditioned by tonic muscle vibration and by cutaneous afferent stimulation.

A pilot experiment has been started this year on tracing physiologically identified muscle afferent fibers anatomically, using intra-axonal iontophoretic injection of horseradish peroxidase. Alpha motoneurons of known unit type are injected in the same experiments.

Major Findings:

A. Discharge patterns of ankle extensor motoneurons when conditioned with cutaneous afferent input.

During FY 1977, we completed a project designed to examine the patterns of MG and soleus motoneuron discharge when conditioned with a particular set of cutaneous afferent input. Early work had shown that the same hindlimb nerves, especially those that convey sensation from the skin of the foot and ankle, can, in the unanesthetized decerebrate cat, produce simultaneous inhibition of the slow twitch soleus muscle and excitation of its fast twitch synergist MG. Work in our own laboratory has shown that the pattern of synaptic potentials produced by sural nerve stimulation in MG motoneurons is such as to suggest that the slow twitch MG units may be inhibited while the fast twitch units in the same pool are excited by sural afferent input. Providing unambiguous experimental evidence to demonstrate this effect proved to be technically difficult but, ultimately, a research strategy was found which was successful. This uses recordings from small natural filaments of the MG muscle nerve and computer averaging methods. When the discharge of MG motoneurons is facilitated by MG muscle stretch or vibration, MG motor units fire with a scale of relative thresholds such that the lowest threshold units belong to slow twitch motor units. When electrical or natural stimulation of sural afferent fibers is superimposed on such activity, the low threshold motor axons (inferred on the basis of much previous evidence to belong to slow twitch motor units) slow or cease to fire at the same time that higher threshold motoneuron axons (presumably belonging to fast twitch units) are powerfully activated. Measurement of axonal conduction velocities of the various active motoneurons is consistent with this unit identification. We can conclude that some cutaneous afferent fibers preferentially activate fast twitch motor units within the MG motor unit pool, a pattern of synaptic organization that is qualitatively different from all other synaptic systems so far investigated. This result suggests that at least two general patterns of organization of synaptic input to these motoneurons exists, and the ordering of motor unit recruitment under different conditions probably depends on the balance of input in the two systems. This possibility should be taken into account in future models of CNS motor control.

B. Control of cutaneous excitation of MG motor units from supraspinal structures.

In view of the above demonstration of differential control of fast and slow twitch motor units from skin afferents from the distal leg in the cat, it is necessary to investigate whether the responsible pathway, which involves interneurons in the spinal segment, is also under the control of fiber systems descending from the brain. The nature and sources of such descending control are important to hypotheses about how the CNS controls the usage of the different motor unit types known to exist in most limb and trunk muscles. This problem has been studied using intracellular recording from identified motoneurons in the spinal cord. Synaptic potentials (especially the excitatory components) produced by sural nerve stimulation are conditioned by volleys in separate descending fiber systems, generated by electrical stimulation of restricted regions of the brain stem or motor cortex. Stimulating electrodes are placed under stereotaxic control into brain stem nuclei (mainly the vestibular complex and the red nucleus, thusfar) or into the motor cortex and/or pyramidal tract. Electrode locations are checked histologically after each experiment. The evidence to date suggests that the excitatory cutaneous pathway from the sural nerve to MG motoneurons does not receive important convergence from vestibular nuclei or the red nucleus efferents, but is excited in some cases from the corticospinal tract. The data thusfar are preliminary and work on this problem will continue during the next fiscal year.

Associated with this problem is a beginning attempt to identify the individual spinal cord interneurons in the sural nerve excitatory pathway, using double microelectrode techniques and spike-triggered computer averaging of synaptic events. These technically demanding experiments have been hampered so far by equipment problems which will hopefully be solved by a newly installed grounding system. This phase of the work is a long-range study and will continue during the next fiscal year, building upon a now-completed anatomical study (using peroxidase tracing methods) of the location of candidate interneurons that project directly to the MG motor nucleus.

C. Anatomical study of physiologically identified muscle afferent fibers.

Considerable work has been done in this laboratory in the past on the physiological characteristics of the synaptic connection between primary muscle spindle afferent fibers (group Ia afferents) and the various types of MG motor units. A number of important questions have been raised by these results which cannot be further elucidated using physiological methods alone, including the soma-dendritic distribution of terminals from one axon and the number of terminals in relation to motoneuron size. Thus, we have initiated a pilot study designed to provide the necessary anatomical information about group Ia afferent fibers. These can be identified by their physiological response to muscle stretch and vibration and then penetrated with an intra-axonal micropipette filled with a solution containing horseradish peroxidase (HRP). HRP has been successfully ejected into individual afferents iontophoretically and, under favorable circumstances, diffuses to label the terminal arborization of that afferent. Motoneurons of identified motor unit type can be labeled intracellularly in the same preparations. While obtaining the type of preparations necessary to answer the outstanding questions will be difficult, we are hopeful that some further insight will be obtained into the anatomical substrate for the Ia afferent-motoneuron relation from this approach.

Significance to Biomedical Research and the Program of the Institute:

Active movement of mammals in space is accomplished by motor units with motoneurons located in the spinal cord. Analysis of the central nervous system control of movement requires a detailed understanding of the organization and interaction of input systems to the spinal cord segments, both from peripheral afferent sources and from supraspinal structures. There is now considerable evidence for the existence of functional specializations among the muscle fibers of different motor unit types, indicating rather precise patterns of motor unit "usage" during movements of various sorts. The long-range goal of the present project is to analyze the patterns of neuronal organization present in the spinal cord as they relate to motor unit type in order to further our understanding of how motor units, and therefore movements, are controlled. Such studies are of clear relevance to analyses of both normal and abnormal movement patterns in man and bear importantly on the interpretation of results of clinical neurophysiological investigations in normal human subjects, and in patients with neurological diseases.

Proposed course of the project:

The investigation of descending systems that project to the excitatory sural afferent pathway will continue, with especial concentration on corticospinal mechanisms and on brain stem pathways not yet explored (e.g., medial reticulospinal and tectospinal). The method of conditioning synaptic potentials being used for this study is now well worked out and we anticipate completing this phase of the work in the next fiscal year. The study of functionally identified interneurons in the spinal cord will continue with special emphasis on the excitatory sural pathway and we anticipate that this will be made somewhat easier when we know more precisely which descending systems project to such interneurons. Preliminary experiments have shown that the basic approach being used is feasible although there are many technical difficulties. We intend to continue attempts to label identified afferents and motoneurons with HRP and perhaps to obtain material suitable for electron microscopy as well.

Keyword Descriptors:

Motoneurons
Motor units
Spinal cord
Interneurons
Synaptic potentials
Neural circuits

Publications:

Burke, R.E., et al. Relative strength of synaptic input from short-latency pathways to motor units of defined type in cat medial gastrocnemius. *J. Neurophysiol*. 39: 447-458, 1976.

Burke, R.E.: The physiology of alpha motoneurons and their synaptic input in relation to the problem of amyotrophic lateral sclerosis. In Andrews, J.M., Johnson, R.T. and Brazier, M.B. (Eds.): *Amyotrophic Lateral Sclerosis: Recent Research Trends*. New York, Academic Press, 1976, pp. 119-133.

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Kanda, K., Burke, R.E. and Walmsley, B.: Differential control of fast and slow twitch motor units in the decerebrate cat. *Exp. Brain Res*. In press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01687 09 LNLC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

TECHNIQUES FOR MAKING CONNECTIONS WITH THE NERVOUS SYSTEM.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Martin Bak, Electronics Engineer, LNLC, NINCDS

Other Investigators:
Robert E. Burke, M.D., Chief, LNLC, NINCDS
George Dold, Electrical Engineer, LNLC
Seth Goldstein, Ph.D., DRS, BEIB
F. Terry Hambrecht, M.D., Fundamental Neurosciences Program, NINCDS
Gerald E. Loeb, M.D., Medical Officer, LNLC
William B. Marks, Ph.D., Research Physiologist, LNLC
Joan S. McIntosh, Physiologist, LNLC
Edward M. Schmidt, Ph.D., LNLC
Bruce Walmsley, Ph.D., LNLC

COOPERATING UNITS (if any) Behavioral Biology Branch, NICHD; BEIB, Division of Research Services, NIH; Fundamental Neurosciences Program, NINCDS; Johns Hopkins University; Clinical Neurosciences Branch, NINCDS; Columbia Presbyterian Medical Center

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bldg. 36, Rm. 5A29, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.6	1.3

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is intended to develop techniques for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in acute and chronic neurophysiological preparations.

Project Description:

Objectives: Successful monitoring of neural activity from the peripheral or central nervous system requires the development of techniques as they apply to unique recording situations. These include both acute and chronic preparations in which stable and discriminable single unit activity is required, including animal preparations that are awake, comfortable and either free to move or moving with minimal restraint.

Methods Employed:

A. Materials suited for biological implantation.

The evaluation of the physical properties and biocompatibility of Parylene-C, iridium and certain medical grade silastic rubbers has continued.

B. Designs for a chronic recording intracortical microelectrode.

1. "Map pin" electrodes have been routinely used in acute experiments in order to record from neurons in the spinal cord of cats. Sufficient data has been accumulated on electrode parameters so that they may now be incorporated in chronic studies.

2. Histological evaluation of neural tissue after electrode implantation is continuing, primarily in monkey.

3. "Hair brush" electrode arrays containing three or more electrodes have been successfully built and implanted. Further testing of the arrays will be performed pending an appropriate experimental design requirement.

4. "Map pin" electrodes have been successfully implanted in the motor cortex of cat for kinesiological studies on the treadmill.

C. Microelectrode introducer capable of tracking the moving brain.

The introducer, a device developed previously and described in the 1975 Annual Report will be sent to another laboratory for studies in the visual cortex of cats.

D. Chronic single unit recording from dorsal root ganglion.

The original electrode system has been modified with favorable results. The present system consists of 7-10 50 micron diameter 90% platinum-10% iridium alloy wires with Pyre-ML (polyimide) insulation. The wires are threaded through a thin-wall silastic tube leading from a connector saddle on the cat's back to a strain relief anchoring to the L7 dorsal spine. The wires are trimmed obliquely and inserted by hand into the L7 ganglion via a small laminotomy at the base of the L7 spine, leaving a loop of wire to take up relative motion during walking.

E. Development of specialized substrates for nerve and muscle tissue culture.

Glass coverslips are coated with 700 angstrom layers of Parylene-C. The surface which is normally hydrophobic can be modified to any degree of hydrophilicity, producing any desired degree of cellular adhesion.

F. 10-Channel high impedance amplifier system.

A 10-channel amplifier system with a small remote active head stage has been developed within the laboratory without having to recruit expensive outside assistance for hybridization. The system has been designed to record from high impedance sources and can be mounted to a connector on a cat's back or monkey's head for chronic studies in which the animal is free to move.

G. Implantable strain gauge for monitoring muscle tension from associated tendon.

A miniature semiconductor strain gauge which is glued to a small uniquely designed stainless steel substrate has been implanted in cat. The intact tendon is fitted onto the device which then measures absolute forces generated by the muscle during normal animal movements.

H. Analog delay for on-line confirmation of neuroelectric signals.

An analog delay has been improved with the use of a hybrid output filter and a L.E.D. readout system in which the precise delay time is continuously displayed.

I. Length gauge

Joint angles from hip, knee, and ankle are simultaneously available as signals from chronically implanted length gauges about those joints in cat hindlimb. Each gauge consists of a 5 cm length of highly elastic Silastic tubing filled with saline at 5X physiological concentrations and plugged at each end with a platinum electrode. The ends are fixed to bone sites via screws or ties. Stretching of the tube during movement results in a resistance change between electrodes which is detected by an AC bridge. The hypertonic saline becomes osmotically pressurized by diffusion of water through the Silastic, reducing spurious kinks or compression.

J. Window discriminator for neural spike sorting.

A time amplitude window discriminator has been improved and will reliably separate a single spike waveform from a multiunit recording, in many cases even when two spikes have the same amplitudes.

K. Chronic EMG recording from muscle.

EMG electrode designs are being assessed and modified in the hope of producing an electrode which will permit detections of single motor unit activity in the hindlimb muscles of cats performing a range of activities. It is hoped to combine this project with the use of the implantable strain gauges in order to observe the use of motor units during normal movement.

L. Self-sealing micropipette.

Glass micropipettes for intracellular neural recording are coated with about 100 Å Parylene-C and then beveled to yield sharp tips with a slippery, hydrophobic shank to promote sealing of the pipette to the cell membrane at the penetration site. Preliminary results in tissue culture have been encouraging.

Major Findings:

Parylene-C having previously shown to be relatively non-toxic histologically has demonstrated that its presence within 100 microns of viable neurons in monkey motor cortex is well tolerated by the fact that at least two electrodes have recorded single unit activity for over fifteen months. This fact further supports the non-histotoxicity of pure iridium, the core conductor of the "map pin" electrode.

"Map pin" electrodes have recorded single unit activity in cat motor cortex for over one month. A significant observation when implanting the "map pins" in cat cortex is the necessity of having to glue the electrodes to the pial surface or they will consistantly pull out of the cortex. This does not appear to be a requirement in monkey motor cortex.

Two 10-channel high input impedence recording amplifiers with miniature active head stages have been built and have satisfactorily recorded signals from monkey motor cortex and from the dorsal root ganglion of cat while the cat was walking and running on a treadmill.

Parylene-C has been found to be extremely non-reactive in a study in which eight cats were implanted with "map pin" electrodes insulated with glass or Parylene. The tissue reaction to Parylene after one year in situ (cerebral cortex) compared favorably to the reaction to glass-coated electrodes after only one month in place.

Modified "map pin" electrodes have recorded single unit activity in the spinal cord of cat for up to three weeks.

Significance to Biomedical Research and the Program of the Institute:

The successful development of techniques for recording signals from the nervous system is essential to the success of ongoing experiments in the laboratory. These newly developed techniques are also beneficial to other laboratories involved in neurophysiological research and may have an impact in the development of prosthetic devices for the neurologically handicapped.

Proposed Course of Project:

Continued modification of the "map pin" electrode for application in other structures of the central nervous system such as the cerebellar cortex is anticipated. Also, studies designed to optimize the electrodes' performance for indefinite periods of time will be conducted with regard to histotoxicity of electrode materials and electrode to cell migration. Electrode arrays for peripheral nerve and dorsal root ganglion will be used to study neural activity with regard to locomotion in awake animals. Plans to attach the strain gauge assembly to all three tendons associated with the triceps surae muscles in cats is anticipated to determine the correlation of integrated EMG to muscle tension. Some of the projects discussed need further development and work will continue on them. Further cooperation with the Neural Prosthesis Program is anticipated.

Keyword Descriptors:

Chronic single unit recording
Microelectrode
Hybridization
Semiconductors strain gauge
Materials testing
Length gauge

Publications:

Schmidt, E.M., Mutsuga, N., McIntosh, J.S., Kanda, K. and Goldstein, S.R.: Intracellular recording from pulsating cerebral cortex. Electroenceph. Clin. Neurophysiol. 42: 581-583, 1977.

Bak, M.J. and Schmidt, E.M.: An improved time-amplitude window discriminator. IEEE Bio. Med. Trans., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01688 09 LNLC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

CORTICAL MECHANISMS OF VOLUNTARY MOTOR CONTROL

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Edward M. Schmidt, Ph.D., Biological Engineer, LNLC

Other Investigators: Martin Bak, Electronics Engineer, LNLC
Robert E. Burke, M.D., Chief, LNLC
George Dold, Engineering Technician, LNLC
L. Durelli, M.D., Guest Worker, LNLC
Joan McIntosh, Physiologist, LNLC

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bldg. 36, Rm. 5A29, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to investigate the behavior of individual neurons in the motor-sensory cortex during the production of spontaneous or operantly conditioned limb movements in awake, minimally restrained animals. Long term chronic recordings from single cortical neurons are obtained with special microelectrodes. The location and coding properties of cortical neurons as they relate to various types of limb movement are being investigated. Micro-stimulation through the electrodes is used to map the output properties of the region of cortex under study. Sensory input is also examined for each neuron by passive manipulation and tactile stimulation.

Project Description:

Objectives: The major goal of this project is to study the behavior of neurons in the motor cortex of primates in relation to spontaneous or conditioned limb movements. This involves the study of relationships between the firing patterns of cortical neurons and the measurable parameters associated with simple movements. One aim is to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to predict the time course and amplitude of various motor responses. This requires a careful study of the types of cells (judged by response characteristics) to be found in the motor cortex, their frequency of occurrence, location and specific function.

Methods Employed: Monkeys were initially trained to move a handle that positioned a cursor light to one of eight spots. The monkey was required to match the position of this cursor light to one of eight target lights. When a match occurred for a prescribed length of time, a juice reward was delivered to the monkey. After implantation of up to a dozen "map-pin" chronic microelectrodes in the motor cortex, the animal's cursor light was controlled by the firing pattern of a single cortical neuron. The task was the same as before except the handle was removed and the monkey was then allowed to make free arm movements. Correlations were sought between cortical neuron behavior and the type of movement selected by the animal.

Map-pin electrodes were also implanted in the motor-sensory cortex of cats that had been trained to walk on a treadmill.

Major Findings:

1. Monkeys are capable of finely controlling the firing rate of most cortical neurons studied, through operant conditioning techniques. Neurons that can be so conditioned are usually associated with discrete limb or finger movements. The monkey tends to continue to improve control over the firing pattern of the contingent neuron the longer the cell is operantly conditioned. The maximum information outflow rate obtained in an eight target tracking task has been 2.5 bits/sec. For comparison the best performance achieved, doing the same task, with a manipulandum and a wrist flexion-extension task, was 6.6 bits/sec. When a new neuron was conditioned the monkey rapidly learned the task that was required to activate the cell.

2. Micro-stimulation through the map-pin electrodes has provided a means of determining the muscles affected by stimulation of specific cortical sites. Both proximal and distal muscles can be activated by trains of stimuli at current levels below 20 μa. Simultaneous excitation and inhibition of EMG activity has been observed. When the neuron recorded by the map-pin electrode was conditioned, the muscle activated was the same as that activated by micro-stimulation.

3. Single unit recordings have been obtained for over 15 months from the precentral cortex of one monkey. One particular cell has been identified and conditioned for more than 2 months. These results indicate that the electrodes and insulation are bio-compatible at least to the extent that neurons close to the electrode remain viable for long periods of time.

4. Chronic recordings have been obtained from freely moving cats with the map-pin electrodes. Thirty two units have been studied during locomotion, natural stimulation, and antidromic pyramidal tract stimulation. All but two units had restricted sensory activation, usually from deep receptors. The other two were activated by touch, deep pressure, and joint rotation.

Proposed Course of Project: Work will continue in the area of obtaining long-term chronic recordings from cortical neurons. The main emphasis of the project will be on obtaining a better understanding of the function of cortical neurons in normal movements. Conditioning of single cell activity will be combined with videotape monitoring of associated limb movements. Pyramidal tract stimulation will be employed to identify neurons projecting into the spinal cord. Micro-stimulation through the map-pin electrodes will be used with EMG recordings to determine the muscles influenced by a specific cortical site. Mapping of sensory input to each neuron studied will also be employed. By being able to characterize the activity of a cell under several different conditions we should be able to better characterize the coding properties of movement-related motor cortex neurons.

Significance to Biomedical Research and the Program of the Institute:

Through our studies we are obtaining a better understanding of the function of the motor cortex in relation to the generation and control of voluntary movement. The "map pin" electrodes have provided single unit recordings from the same neuron for many months which make feasible studies on movement under a variety of conditions. Micro-stimulation through the electrodes combined with EMG studies provides a means of mapping the output region of the section of cortex under study. This work also has significance for the development of cortically-controlled prosthetic devices, although the electrodes are not yet satisfactory for obtaining prosthetic control signals where recordings for a number of years are required.

Keyword Descriptors:

Chronic recording
EMG recording
Micro-stimulation
Motor cortex
Operant conditioning
Single-unit recording

Publications:

Schmidt, E.M., Mutsuga, N. and McIntosh, J.S.: Chronic recording of neurons in epileptogenic foci of monkey during seizures. Exp. Neurol. 52: 459-466, 1976.

Schmidt, E.M., Bak, M.J. and McIntosh, J.S.: Long-term chronic recording from cortical neurons. Exp. Neurol. 52: 496-506, 1976.

Schmidt, E.M., Bak, M.J., McIntosh, J.S. and Thomas, J.S.: Operant conditioning of firing patterns in monkey cortical neurons. Exp. Neurol. 54: 467-477, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02079 04 LNLC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

MODELS OF NEURAL INTERACTIONS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: William B. Marks, Ph.D., Research Physiologist, LNLC, NINCDS

Other Investigators: John A. Hodgson, Ph.D., Visiting Fellow, LNLC
Bruce Walmsley, Ph.D., Visiting Fellow, LNLC

COOPERATING UNITS (if any)

None

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Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bldg. 36, Rm. 5A29, NIH, Bethesda, Maryland 20014

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1.2	1.1	0.1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. The change in passive membrane polarization of cells, axons, and dendrites beneath a cortical surface stimulation electrode has been calculated at a variety of shapes, orientations, and depths.

2. The method of third moment factor analysis described in the last report can now be applied to data with intrinsic variance. Thus it now appears possible to calculate independent linear combinations of empirical signals.

Project Description:

Objectives:

1. To develop a method for calculating the pattern of membrane depolarization in nervous tissue caused by an applied pulsed field. To explain the surprising ability of surface cathodal stimuli to fine pyramidal cells directly.

2. To develop a method for determining the number and time course of the underlying variables in an array of correlated empirical signals.

Methods Employed:

1. The passive properties of neuronal membranes and myelin in response to pulsed extracellular fields are modelled as a linear partial differential equation in one variable. Dendritic branches at various angles, axon terminals, and somas with a limited variety of shapes can be modelled in this way. The equations are solved directly rather than iteratively, and use only 1/10 as much computer time.

2. For n data variables, the n x n correlation matrix and the nxnxn third order correlation array are computed. The required number of samples should be a certain function of the number of underlying variables and their skewness. The correlation matrix is factored by a standard method, and the result is modified in relationship to the third order array to yield a matrix L. This is used to linearly combine the data into a smaller number of new independent variables.

Major Findings:

1. Surface current pulses of a strength duration and density known to cause phosphenes or movements were simulated. Anodal pulses should directly fire all somata within 1 mm of the electrode, 1 μm horizontal axons within .25 mm, and 1 μm vertical axons terminating within .5 mm of the electrode. For similar cathodal pulses, horizontal and vertical axons within .5 mm of the electrode, and larger axons at greater distances, would have to mediate the firing of somata. Ascending recurrent axon callaterals could fire somata non-synaptically at about the same threshold for pulses of either polarity. Thus ascending collaterals might mediate the direct cathodal responses.

2. Empirical data, which is measured with error, is now acceptable by the equations used to compute the independent linear combinations of correlated variables.

Significance to Biomedical Research and the Program of the Institute:

1. It is now possible to calculate the polarization change along a rather complicated neuron with branching dendrites and myelinated branching axon, caused by an applied extracellular field of arbitrary spatial and temporal shape. The results for a surface electrode provide a background for understanding the responses to surface stimulation in the visual prosthesis program and for motor cortex stimulation experiments.

2. Much of the scientific method consists in seeking the simplest underlying structure to explain an array of related observations. Perception depends on feature detectors, which appear to measure the independent aspects of sensory patterns. The notion of independent sources appear fundamental for both of these. Independent sources are functions of the data which are independent. They are candidates for important underlying variables in the system under study. The present theory only deals with linear systems without time delay, but it and other methods provide an increasingly general methodology for automatic detection of the underlying structure in data. The present theory is now perhaps of interest whenever large amounts of parallel data are produced, as in neurophysiology, applied physics, and economics.

Proposed Course of Project:

1. A collaborator interested in stimulation of the cortex or cerebellum is now being sought, who could help test the predictions in a specific situation.

2. The ability to deal with data having intrinsic variance may now allow us to calculate the independent features of visual patterns. This project was begun in the summer of 1975 with the help of Richard Blum but required the present improvement before it could continue.

However the main interest is in applying the notions of multiple data channels to the activity of motor units in the EMG. EMG records from an active muscle recorded by a monopolar lead or a differential pair are usually influenced by several motor units which cannot be reliably distinguished. It is proposed to separate these multiunit records into several single unit records by recording from the same region of the muscle with several leads. The unit signals would be distinguished by differences in the pattern of amplitude and waveform that each signal has among the several leads. The derived unit records would be used singly to determine how motor units of the three known types are used in unpredicted ways to assist in various types of movement, and how the different descending systems of the cord influence the three types differently. However the principal investigator is mainly interested in studying the derived motor unit activities simultaneously, in order to determine how many signals would have to be applied to the motoneuron pool simultaneously to account for the observed number of degrees of freedom of the pattern of activity. Thus if recruitment order were always determined by the same inferred rank order and if the activity after recruitment always increased in a way that could be derived from a single underlying variable for all the neurons, the activity of the pool would be governed by a single underlying variable. Possibly however a single variable will suffice to predict the activity imperfectly, while others would be required to account for relatively minor variations in the pattern for different movements or perhaps each type of motor unit is governed by a single variable, so that three variables would be required for the entire population. Such questions would be much easier to answer if the simultaneous activity of several motor units were available.

In preliminary experiments we confirmed that it is unusual to record single units with single or differential leads, but a series with multiple leads was encouraging. Six leads about 2 mm apart recorded several units each, but each unit appeared to have a distinguishable distribution of amplitudes across the several leads.

We propose to first seek a configuration of electrode placements and suitable muscle, such as the medial gastrocnemius of the cat, on which the various motor units exert a distinguishable pattern of amplitudes and waveforms. If it were possible to place more electrodes in a given region than the number of major motor units in that region, then in theory all these uni could be sorted from one another even when they are all simultaneously acti in a relatively simple way. This number is of the order of 15 to 30, presumably.

We would then attempt to distinguish the units by their pattern of amplitudes into the leads, without utilizing waveform differences. This would greatly simplify the analysis, and would also allow us a relatively brief portion of the wave, so that temporal overlap of the unit events woul be less common. The rate of rise of the EMG wave at its maximum point is the best candidate for a brief amplitude measurement.

Third, since the use of motor unit data requires many thousands of distinguished events, it will be necessary to automate the unit recogniti process. If the configuration of briefly sampled wave amplitudes suffice characterize the units, then they might be separated as follows. For nonsimultaneous motor unit firings ('events') the computer could consult table of clusters of previously recorded configurations of amplitudes in several leads, with perhaps relative delays if these are selective. If a large unclassifiable event occurs it would be tested as a possible coincide This might be done through a previously computed matrix which would linea combine the lead signals into an equal number of signals which would have minimal statistical relatedness ('independent sources'); each derived si might contain a non-overlapping subset of the population of units. Then would be likely that the coinciding events would occur separately in th derived relatively independent signals. Since the units that produced the amplitudes registered in the past would be known, the possible pairs could then be quickly compared with the actual event.

If waveform information is also required to distinguish the events, a simple addition to the amplitude-oriented technique will be sought, such as sampling the still unclassified events at a second time, at a certain delay after the amplitude was measured. This might provide a distinguishing measurement.

It is likely that we would be interested in investigating the anatomy of the motor units, to get a clearer picture of the manner in which the units overlap in space. This might suggest better ways to place the leads. The electrical data should give information about the distribution within the muscle of each motor unit.

Keyword Descriptors:

Pattern recognition
Motor units
Multineuronal

Publications

Marks, W.B. and Loeb, G.E.: Action currents, internodal potentials, and extracellular records of myelinated mammalian nerve fibers derived from node potentials. Biophys. J. 16: 655-668, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02080 04 LNLC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

NEURON ACTIVITY DURING LOCOMOTION.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Gerald E. Loeb, M.D., Medical Officer, LNLC, NINCDS

Other Investigators:
Jacques Duysens, M.D., Ph.D., Visiting Fellow, LNLC
Bruce Walmsley, Ph.D., Visiting Fellow, LNLC
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COOPERATING UNITS (if any)

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Laboratory of Neural Control

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new technique is being used to monitor at the unit level the primary afferent neural activity entering the spinal cord during normal walking. Approximately 10 fine wires are implanted in the L7 and S1 dorsal root ganglia (DRG) of a cat. After recovery from surgery, each wire usually records separable unit activity from 1-3 DRG cells, and each unit is typically stable for 1-4 days. This permits correlation of unit activity with kinesiological records of normal and perturbed gait as well as accurate characterization of receptor modality under anesthesia. 67 units covering the entire range of unmyelinated and myelinated afferents have been so studied and characterized to date. Preliminary results indicate some previously unpredicted activity patterns which will require further study.

Neural and electromyographic activity is being correlated with records of muscle force and joint angle from chronically implanted strain and length gauges developed for this project. In particular, the forces and EMG output of individual muscles of triceps surae have been studied during walking, running, and jumping in implanted cats.

Project Description:

Objectives: The major goal of this project is to examine directly the roles of spinal neurons and primary afferent fibers in normal movement which up till now have been inferred from paralyzed or decerebrate acute preparations. The principle current emphasis is on natural patterns of afferent and efferent activity in the spinal cord and the role of afferents as part of a system of servo-mechanisms. Spinal reflexes elicited by both cutaneous and proprioceptive afferent activity are now known to have a profound effect on locomotory patterns.

Methods Employed:

1. The present method for obtaining stable, unit records during normal walking consists of inserting insulated 50 micron diameter wires into the DRG via a small laminotomy. The cut ends of the wire constitute the recording surface and the only fixation is by a flexible Silastic carrying sleeve sutured to the dorsal spinous process.

2. Supporting kinesiological measurements include continuous read-out of joint angle at ankle, knee, and hip via implanted length gauges of our design, EMG from selected muscles, and videotape of gait.

3. Computerized analysis of unit activity and correlation with kinesiological variables is presently under development on the PDP-12 computer. Hardwired devices for inter-spike-interval calculation, EMG integration, and sophisticated spike discrimination have been implemented.

4. The forces produced by individual muscles (medial gastrocnemius (MG) and soleus (SOL)) in the hindlimb of freely moving cats have been measured in the respective tendons of insertion using chronically implanted devices. The electrical activity (EMG) of these muscles was also recorded.

Major Findings: Sixty-seven DRG units from eleven cats have been recorded during unrestrained treadmill walking and subsequently characterized by physiological tests under anesthesia. The entire range of myelinated and unmyelinated afferents (0.8 to 108 m/sec) appears to be sampled by this technique, yielding the following preliminary observations:

Of 18 hair receptors (40 to 94 m/sec), almost all had highly repeatable firing patterns during walking even when their fields were not contacted by objects (e.g. on dorsum of foot or lateral hip).

14 light touch receptors (27 to 87 m/sec) were generally inactive unless their receptive fields contacted an external object. Skin stretch sensitivity appeared to modulate or initiate activity in some but not all of 4 such stretch sensitive units.

11 spindle primaries (70 to 105 m/sec) and 4 spindle secondaries (43 to 57 m/sec) generally responded to passive stretch and to an apparently variable alpha-gamma coactivation. Some units had only one activity peak during passive stretch. Others had an additional peak during active muscle contraction. This latter activity could reach frequencies above that of the passive stretch peak but usually showed a pause during maximum shortening rate of the muscle. In our scattered sample, the only systematic difference between primaries and secondaries appeared to be higher peak firing rate in some but not all primary endings.

4 Golgi tendon organs had activity closely correlated with EMG activity and, presumably, active tension of their muscles, with very little passive stretch response during gait or manipulation.

5 knee joint receptors (46 to 75 m/sec) had very complex and variable activity patterns not simply related to joint angle, and were possibly influenced by joint loading and/or capsular muscle tension. Units with similar responses to manipulation could have very different activity during walking.

There were 10 units of undetermined modality, often with complex bursting and/or long periodic cycles of spontaneous activity usually not modulated by gait. One unit was modulated by blood pressure changes, but others have been unresponsive to a variety of thermal, noxious, and visceral stimuli, and may represent the poorly understood classes of free endings and/or sympathetic afferents.

The strain gauges demonstrate that SOL produces virtually the same peak force (1.5-2 kg wt) over the entire range of hindlimb action (from quadripedal standing to 1.2 m vertical jumps) and, contrary to some previous assumptions, actually plays a major role in locomotion. In contrast, the peak forces from MG vary more widely over the same range of movements, from less than 500 gm-wt in standing to 10 kg-wt in jumps. Peak MG force was clearly graded with speed of walking while that from SOL was not. The difference in the range of movements over which peak MG and SOL forces are graded is very likely related to the differences in motor unit populations making up these two muscles. The data also gives insight into which motor unit types are likely to be active in the MG muscle during a particular movement.

Significance to Biomedical Research and the Program of the Institute:

Much current work on the study of mammalian locomotion has been concentrated on the cat hind limb, where considerable knowledge is already available concerning the physiological and anatomical properties of the muscles, motor neurons, afferents, and spinal reflexes. However, the details of the functioning of this system during normal locomotion under cerebral control can at present only be inferred, giving rise to a number of competing control theory hypotheses. The new methods employed in this project should provide data needed for testing such hypotheses and formulating new ones. An understanding of the normal control of movement is essential to understanding a number of degenerative diseases of the spinal cord (e.g. ALS, transverse myelitis, etc.) affecting locomotion. A longer range application of the technique of chronic transducers and afferent monitoring is in the field of functional neuromuscular stimulation (FNS). Sophisticated devices designed to restore motor function by bypassing CNS lesions (e.g. paraplegics) will probably require some form of closed loop servo-control utilizing transducers of muscle length and tension and of skin pressure. If it proves possible to obtain stable afferent activity or implanted transducer signals over long periods of time, such devices could improve the function and simplify the design and implantation of complete FNS systems.

Proposed Course of Project:

Research activity for the next year will concentrate on acquiring a larger sampling of afferent units from each modality and further exploring their sensitivity to detect applied perturbations to the gait (electrical and mechanical stimuli, grade and speed changes, etc.).

A long term research goal is to detect principles of organization among receptor types and the information content of their activity patterns during locomotion. Ultimately, it is hoped to correlate this organization with the organization of central projections and reflex pathways.

A long term technological goal is to determine the limits of recording stability and tissue compatibility in both time and numbers of information channels with a view to assessing the feasibility of deriving somatosensory information for the feedback control of Functional Neuromuscular Stimulation Prostheses.

The study of efferent activity patterns is being directed towards obtaining information on single motor unit activity. Preliminary work has begun on two independent methods: separation of single units from EMG electrodes and implantation of "floating map pin" microelectrodes to record from single motor neurons in the spinal cord. Either or both of these methods will eventually be combined with implanted transducer measurements of tendon force and length, and possibly nerve cuff or DRG records of simultaneous afferent activity.

Keyword Descriptors:

Dorsal root ganglia
Locomotion
Chronic recording
Spinal cord
Somatosensory activity
Kinesiology

Keyword Descriptors (Cont'd)

Muscle force
Strain gauges

Publications:

Loeb, G.E., Bak, M.J. and Duysens, J.: Chronic unit recording from somatosensory neurons in the spinal ganglia of the freely walking cat. Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02160 03 LNLC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

INTRINSIC PROPERTIES OF MOTOR UNITS.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Robert E. Burke, M.D., Chief, LNLC, NINCDS

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COOPERATING UNITS (if any) Dept. of Neurology, Univ. of Maryland School of Medicine, Baltimore; Dept. of Neurosurgery, VA Hospital, Syracuse, NY; LNNS, NINCDS; Dept. of Physiology, Univ. of Alberta, Edmonton, Alberta, Canada

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1.7	1.3	0.4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to provide information on the ranges and distributions of the electrophysiological and morphological characteristics of alpha motoneurons and of the interrelated mechanical, histochemical and morphological properties of the muscle fibers innervated by them (i.e., the muscle unit). The motor unit populations in normal animals are compared with those in animals after various conditioning treatments.

PHS-6040

Project Description:

Objective: This project is designed to provide information about the populations of motor units that make up large limb muscles in mammals, primarily the cat. Research involves the study of the electrophysiological and morphological characteristics of motoneurons in relation to the mechanical, histochemical and anatomical properties of the muscle units innervated by them. The neural and muscular elements are functionally inseparable and they are therefore considered together. The characteristics of normal motor unit populations are studies as well as alterations in motor units produced by changes in mechanical demand, central nervous system lesions or other experimental manipulation.

Methods Employed: Analysis of motor unit populations is carried out in anesthetized (Halothane and/or pentobarbital) cats, using intracellular stimulation and recording in individual alpha motoneurons to ensure functional isolation of single motor units. The electrophysiological properties of the innervating motoneurons (as well as the organization of input synaptic information) can be examined using conventional electrophysiological techniques and then the mechanical properties of the innervated muscle unit is assessed by recording tension production at the appropriate tendon during stimulation of the motoneuron with various patterns of pulses, according to criteria worked out in earlier experiments in this laboratory. The muscle fibers of individual units can be recognized histochemically by the technique of glycogen depletion, as done several years ago in this laboratory. In recent experiments, we have simply examined the histochemical composition of test and control muscles by removing target muscles at the conclusion of motor unit survey experiments, freezing in isopentane cooled to -160° in liquid nitrogen, and subsequent histochemical processing at the University of Maryland School of Medicine.

During the past year, most of the motor unit survey work in this project has involved assessment of the effects of altered demand, or of CNS lesions, on the motor unit population of the medial gastrocnemius (MG) muscle. Preparations tested have included cats with one hind leg immobilized at knee and ankle by steel pins through the respective joints, with survival times of 2 to 24 weeks, and animals with either chronic spinal hemisection or complete trans-section in low thoracic areas, with survival times of 2 - 6 months.

A second experimental approach has used injection of purified horseradish peroxidase, type VI (HRP) into the MG muscles after section of other muscle nerves in the hindleg. After a 3-day survival, animals were perfused with glutaraldehyde-paraformaldehyde fixative under deep anesthesia. The spinal cord was processed in these animals for electron microscopy after preliminary examination in the light microscope of fresh, thick-sectioned material processed for demonstration of HRP.

Major Findings:

A. Motor Units after Chronic Immobilization.

The study of the response of MG motor units to chronic (2 - 24 weeks) joint immobilization was begun during FY 1975 and completed during FY 1977. A total of 10 experimental animals were used and the data drawn from these were compared with data from 19 normal animals, obtained in earlier experiments. The use of normal data from a different series of animals was dictated by technical considerations, but the two series of animals were approximately matched for whole body weight.

When compared to normal MG motor units, the units sampled from MG motor pools in cats with immobilized hind limbs showed striking diminution of tetanic tension output under standardized conditions for all four types of units currently recognized. The greatest percentage of changes were found among the more fatigue-resistant unit types (types FR and S) units, as compared with the fatigue-sensitive type FF group. Twitch tensions were also decreased, but not in strict parallel to the changes in tetanic tensions, so that the twitch-to-tetanus ratio, an index of the completeness of contractile element activation, was increased for all unit groups after immobilization. Neither twitch contraction times nor the mechanical properties on which unit typing is based (including resistance to fatigue) were altered significantly in the atrophic muscles. Of particular interest is the observation that the amplitude of the excitatory synaptic potentials (EPSPs) produced by muscle spindle primary afferents (group Ia) in the atrophic MG motoneurons were significantly reduced in all of the type groups of units. The genesis of this change is obscure but it represents an apparent plasticity of a synaptic input system to motoneurons in a situation of chronically-altered mechanical demand.

A complete analysis of the histochemistry and morphology of the atrophic MG muscles (and of the synergist soleus) in the pinned leg as compared to the unoperated contralateral muscles has been undertaken in association with the above physiological studies. The relative proportions of muscle fibers of the three major histochemical types, which correspond to the major physiological motor unit groups, are unchanged after chronic immobilization. However, there is considerable diminution of the diameter and area of individual muscle fibers, consistent with the observed loss in wet weight as compared to contralateral unoperated muscles. In some animals, this fiber atrophy was most dramatic among the fatigue resistant unit fibers (types FR and S), as would be expected from the physiological results. In other animals, the fiber atrophy appeared much more uniformly distributed through all of the unit types. This inter-animal difference was not clearly related to either completeness of immobilization nor to the relative abilities of the animals to compensate for the imposed disability (all animals were videotaped during walking and running on a treadmill, to enable such functional comparisons). Analysis of this data is continuing and should be complete by the Fall of 1977.

Two animals were examined at relatively short intervals after immobilization (20 days and 5 weeks). In both, the same pattern of immobilization changes found in the longer term animals (4-6 months) were found, although quantitatively less than in the long-term animals. Thus, immobilization changes appear soon after the onset of the disability but continue to progress slowly over a period of months.

B. Motor Unit Properties After Spinal Cord Section.

A pilot series of experiments has been undertaken on cats with chronic spinal hemisection, in several cases involving bilateral hemisections of the low thoracic cord staggered in time and in location. These cats displayed classic signs of motor disability and reflex changes but also exhibited a remarkable compensation over a period of several months, so that they moved about with relative ease, despite some exaggeration of stretch reflexes in the hind legs (spasticity). The motor unit population surveys so far undertaken in such animals have failed to reveal any marked alteration in the MG motor unit population, and the histochemistry of the affected muscles similarly shows little change from the normal. Studies of such animals will continue using cats with chronic (6 months) complete spinal section, where there is marked disability in limb control and pronounced reflex changes.

C. Ultrastructural Studies of MG Alpha and Gamma Motoneurons Labeled with HRP.

Building on previous work with HRP labelling of MG motoneurons with HRP injected into the MG muscle, we have undertaken a pilot experiment to gain information about the ultrastructural features of identified alpha and gamma motoneurons belonging to this nucleus. Alpha and gamma motoneurons are distinguishable after HRP labelling not only on the basis of soma size, which is well known, but also because of a systematic difference in the intensity of HRP label. The gamma motoneurons are labeled much more heavily than the alpha, an observation so far unique in the CNS with this tracer substance. This seems due to a higher density and larger size of labeled lysosomes in the gamma motoneuron cytoplasm as compared to the alpha motoneurons. We have obtained material from two cats for electron microscopy and study of this material has now begun.

D. Identification of an Example of Skeletofusimotor (or Beta) Innervation.

We have recently re-examined older material in which individual motor units were studied by intracellular recording and unit muscle fibers were identified histochemically by glycogen depletion, in order to search for examples in which the same motoneuron may have innervated both extrafusal skeletal muscle fibers and intrafusal fibers, i.e., the muscle fibers within the muscle spindles in the same muscle. This search was prompted by recent reports from another laboratory that such dual innervation patterns can be found by the glycogen depletion technique. We have in fact located one such example in our own material. This example, from a soleus muscle, showed that the electrophysiological properties of the innervating beta motoneuron, and the mechanical and histochemical properties of the innervated extrafusal muscle unit, were little different from those found in other, presumably pure alpha, motor units. Furthermore, this example is important in that it demonstrates evidence that primary muscle spindle afferents made contact with the beta motoneuron (i.e., produced a monosynaptic EPSP), which is a connection never observed in pure gamma motoneurons. This would represent a positive reflex feedback loop and the finding assumes some importance for control theories involving spindle feedback in view of the recent and growing evidence that beta innervation is not rare in higher mammals, even in relatively large limb muscles.

E. Motor Unit Studies After Chronic Nerve Section and Re-innervation.

A pilot series of experiments has been started in collaboration with the Dept. of Physiology, University of Alberta, in which motor units are studied in muscles after the target muscle nerve has been cut and allowed to re-innervate the same muscle. The animals involved were monitored for functional regrowth of motor and sensory axons by chronically-implanted nerve cuff electrodes, using methods developed at the University of Alberta. Preliminary analysis of the data from these recent experiments indicates that motor units reorganize such that their properties are not very much different from those in normal muscles, but there are also changes in motor axon conduction velocity that persist for long periods after successful re-innervation. These results, if confirmed by further study, will have importance to clinical problems of nerve injury and regeneration.

Significance to Biomedical Research and the Program of the Institute:

Analysis of the control of movement by the central nervous system requires consideration of the properties and functional specialization of motor units, as these are the quantal elements from which all skeletal movements are composed. Study of the interrelation between the intrinsic properties of motor units, including both the motoneuron and muscle unit portions, and the organization of synaptic input to the same units has aided our understanding of the control problem and has suggested new avenues for research. In addition, elucidation of the detailed interrelation between the physiological, morphological and histochemical characteristics of muscle units in animal muscle has immediate relevance to investigations of human neuromuscular disease, in which electromyography and muscle histochemistry play important diagnostic and research roles. There is growing evidence that the basic pattern of motor unit organization in animals and man is similar in principle. In particular, the histochemistry of limb muscles in the cat and in man is remarkably similar. Studies of the effects on motor unit populations of altered usage, CNS lesions, and denervation - re-innervation in the cat have important relations to the interpretation of clinical investigations in patients with neuromuscular disorders, peripheral neuropathies and CNS lesions.

Proposed Course of the Project.

Final analysis of the data from immobilized animals should be complete by the end of FY 1977. The collaborative studies of motor unit populations after spinal cord lesions (hemisection and total section) will continue into FY 1978, with particular attention to animals with complete spinal section for various durations. The methods for obtaining and analyzing data from such experiments are now firmly established and our experience to date indicates that we can recognize important changes from the normal pattern both in the motoneurons and their muscle units. These experiments will be done in collaboration with the Dept. of Neurology, University of Maryland School of Medicine. Whether or not the collaboration with the University of Alberta will continue during FY 1978 depends on final analysis of the results already in hand and on agreement about the considerable logistical problem involved in the long-distance interchange of animals and personnel. Study of the material already in hand from spinal cords containing labeled MG alpha and gamma motoneurons will continue into FY 1978 and may well be completed by the end of that year. Finally, we anticipate that a long-term project to study the effect of fast-slow muscle nerve cross-union between the soleus and flexor digitorum longus muscles will be initiated in FY 1978. We already have some experience with this preparation and it should yield important data on the general area of "trophic" interactions between motoneurons and foreign muscle fibers, using an experimental approach that is much more controlled than any previously attempted in this difficult area.

Keyword Descriptors:

Muscle fibers
Motoneurons
Contractile properties of muscle
"Trophic" effects

Publications:

Burke, R.E., Rudomin, P. and Zajac, F.E.: The effect of activation history on tension production by individual muscle units. Brain Res. 109: 515-529, 1976.

Strick, P.L., Burke, R.E., Kanda, K., Kim, C.C. and Walmsley, B.: Differences between alpha and gamma motoneurons labeled with horseradish peroxidase by retrograde transport. Brain Res. 113: 582-588, 1976.

Mayer, R.F., Burke, R.E. and Kanda, K.: Immobilization and muscle atrophy. Trans. Am. Neurol. Assoc. 101: 1-5, 1976.

Burke, R.E. and Tsairis, P.: Histochemical and physiological profile of a skeletofusimotor (β) unit in cat soleus muscle. Brain Res. In press, 1977.

Burke, R.E., Strick, P.L., Kanda, K., Kim, C.C. and Walmsley, B.: Anatomy of the medial gastrocnemius and soleus motor nuclei in the cat spinal cord. J. Neurophysiol. 40: 667-680, 1977.

Walsh, J.V., Burke, R.E., Rymer, W.Z. and Tsairis, P.: The effect of compensatory hypertrophy studied in individual motor units in the medial gastrocnemius muscle of the cat. J. Neurophysiol. In press, 1977.

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

Henry G. Wagner, M.D., Acting Chief

Unfortunately, illness has prevented the Chief of LNP from continuing the outstanding leadership and participation that the laboratory effort normally enjoys.

Experimental work continued on 10 projects previously initiated. Seven projects were terminated as the objectives were reached and/or the departure of key investigators made further work impractical. The appointment of a number of new visiting scientists to the department brought new skills, and ideas, and made possible a number of fresh approaches. As a result 7 new research tasks were initiated.

In a general sense the research of the laboratory may be conveniently separated into four main areas of investigative effort plus some ancillary thrusts because special opportunities and skills combined to make a particular study appropriate and desirable.

One of the main work areas has been concerned with understanding the initial events in photo-receptor action. This focus of attention is in the forefront of attention today. While we know the photon is absorbed by rhodopsin, there is a substantial ignorance of the subsequent events which intrigues our curiosity. The physical stimulus (whether it is light, pressure or chemical) triggers a complex metabolic pathway which affects the membrane potential. Solving it in photoreceptors will be important to understanding receptor action. LNP studies in the past have looked for similarities in statocysts and hair cells. The photoreceptor has enjoyed primary interest because more is known and the stimulus can be specified more accurately. The prime focus this year has been on the ionic events, the importance of calcium and whether the membrane potential changes produced by photon absorption can be adequately explained by what we know about the calcium, sodium, or potassium concentrations present.

Another main focus of effort has been the elucidation of the relationship of synaptic fine structure and interneural function. The electron microscope has made it possible to analyze and identify the fine structure of interneural contacts in extraordinary detail. Recent advances in marking techniques have allowed us to pinpoint electrophysiological function to a particular cell which we can determine its fine synaptic structure.

Thus looms the vista of an investigative effort which could yield "hard wiring" diagrams in the future. Truly, this is an exciting vista not believable a year or two in the past. As with the photoreceptor, the retina provides this unique opportunity to exploit this concept of combining EM and electrophysiology to determine how neuron structure interrelate in function.

A third area of study recognizes that unique opportunities are provided by simple cell systems such as tissue culture neurons and molluscan neurons which provide simple neural networks, allowing the elucidation of fundamental principles easier than in complex neural centers. Our attention is on the pharmacological actions of certain peptides. These agents appear to act as neurohormones. Again, one of the exciting new areas of study believed to be especially important.

The fourth major area of thrust is concerned with information processing through a complex neural plexus. This is not a new focus of study but it is a most important one and there is a vast field of ignorance remaining. We still do not understand very well how the characteristics of the stimulus are encoded into neural potentials - it is a veritable jig saw puzzle. We have a lot of known information which no one knows what to do with it. New information, even of seemingly trivial import may have implications. Because the retina is a complex neural plexus with distinctive stratification of nerve cells accessible to electrophysiology it lends a unique opportunity to study neural processing.

Our attempts to understand photoreceptor action better, continues to receive attention. One study of ionic mechanisms associated with the phototransduction in rods focussed on the complex potential waveform observed in response to a step increase in light. This study showed that the initial transient was dependent upon the rate of rise of the illumination while the plateau reached later was related to the light intensity. The data seems consistent with the idea that there are two processes, one a voltage dependent process and the other a conductance change associated with the photon flux.

A current hypothesis is that the calcium ion is the activating particle released when a photon is absorbed in the photoreceptor. It has been possible to subject this hypothesis to a critical test in the case of the turtle cone. We used a chelating agent EGTA which lowers the level of free calcium ions. In the dark, conductance increases in accordance with the hypothesis when EGTA is added to the receptor bathing fluid but while bright light increases the response, dim light gives a lower response. High calcium perfusate lowers the conductance but the time course is altered. Since calcium and light have different kinetics, we believe calcium may not be the ion which initiates the membrane response.

It has been known for some time that in the dark, the intrareceptor potential shows fluctuations thought to reflect random ionic movements across the membranes. A study, not yet complete is looking at this "noise" as altered by changes in the calcium concentrations in ionic media. If calcium is the critical ion in phototransduction we hope this study will shed some light on the role of this interesting ion.

Synaptic interactions underly the formation of receptive fields of horizontal cells and bipolar cells. The tiger salamander retina has proven valuable experimentally since it permits good cell penetrations with long lasting recordings. Since it is a vertebrate retina with many of the common general features of organization, it is a suitable model for vertebrate retinas. With the help of a recent major technological breakthrough, the intracellular injection of horseradish peroxidase permits the use of the electronmicroscope to examine fine morphologic detail associated with the particular cells being recorded from and to reveal the synaptic contacts that the cell being recorded from is making. Some of the work has also concerned itself with spectral sensitivity curves which identify the type of input to the horizontal or bipolar cells. This work has gone especially well. We think we can explain why some of the features of the receptive fields of bipolars differ from that of the horizontal cells.

We have mentioned that an area of effort has been the study of certain peptides which appear to act as neurohormones. These substances have actions unlike classical neurotransmitters. For one thing, their time course of action is different; and the threshold action dose is much smaller. But most important is the fact that they have multiple action sites which are reached by diffusion and transport of the active substance through the lymph or blood. These peptides represent a kind of communication system whereby one substance can alter, simultaneously, the activity of a wide variety of target neurons anatomically remote from each other. Vasopressin is a good example of a peptide neurohormone. Molluscan pacemaker neurons offer an unusual opportunity to examine the action of these substances using voltage clamp techniques combined with electrical excitation and iontophoretic injection. We are in the process of determining the action of Lysine-vasopressin on pacemaker conductances.

Another study on molluscan pacemaker neurons has been concerned with the effect of dilantin and other anti-convulsants on their bursting activity. Usually bursting potential are found to be paroxysmal and random, in some aspects this is suggestive of the kind of neuron behavior observed in epileptic foci. On the possibility that this phenomena might offer a convulsive phenomena, we have found it of interest that dilantin abolishes this bursting. We are attempting to identify and characterize the phenomena, measure changes in ionic conductances and catalog the sequence of events.

The cat has also received continued attention as a model for elucidating the way in which neurons interact and process information. Much is known about the cat visual system experimentally so when new facts are found it is frequently possible to relate these quickly to a broad web of knowledge. Research on the cat visual system is one of the fastest moving fields of study. We, in collaboration from the NEI personnel, have found it possible to electronically record and inject into retinal ganglion cells, fluorescent dyes. The close coordination of morphologic detail by light microscopy has made it possible to identify the ganglion cell which evokes "on" type responses (as well as ganglion cell which evokes "off" responses). It was determined that this response characteristics can be related to the interplexiform stratum in which the ganglion cell dendrite ramification takes place. Flat cone bipolars contact the "off center" ganglion cells and invaginating cone bipolars contact "on" center ganglion cells.

Studies carried out in collaboration with investigators at Duke University found good evidence that there is a green cone in the cat retina. Although long suspected of being color blind, persistence by some scientists found unmistakable behavioral evidence for wavelength discriminations. Morphologists, long ago noted that this retina had cones but all were thought to have the same photopigment. We had long been puzzled by some of the old granit data on color modulators. Part of the problem was separating scotopic conditions where rods function, from photopic mechanisms where only cones function. We believe we have found a 500 λ mm max cone functioning. The significance of this is to give the cat a trichromatic visual system, since the blue and yellow-green cones are well established. Far reaching is the possibility that most mammals, once believed to be color blind like cats, have in fact, trichromacy at least in the retina.

Work carried out in 1975 in collaboration with investigators at Canberra is now being reported out concerning some aspects of the role of the lateral geniculate nucleus. This complex structure is an important way station for visual information to the cortex. But is it just a relay station? What sort of processing is carried on? One of the objectives of the collaborative effort was to determine how many of the known classes of retinal ganglion cells send axons to the lateral geniculate. Our study indicated that most of classes, possibly all, do in fact relay through the LGN to the cortex. An opportunity presented itself to look at one aspect of a curious anomaly in Siamese cats. In Siamese cats, substantially more optic axons decussate than in normal domestic cats. Why this is so, is not understood but the anomaly appears to be curiously, associated with the gene that controls the coat pigment. What we found in Siamese cats was that the normally sharp visual vertical meridian is blurred greatly. We found a broad vertical zone in which the ganglion cells, sending axons to the contralateral LGN and others sending to the ipsilateral. Patchyness is not present although predicted on the basis of anatomical studies of the LGN.

One of the terminated projects was concerned with the dichroic microspectrophotometer. This instrument was dismantled at the beginning of this period and transferred with the principal investigator to the Marine Biological Laboratory where work is being continued but will be reported as a part of the Laboratory of Biophysics program.

We can report considerable progress with our rapid scanning microspectrophotometer. Following several years of development to overcome various problems, an extensive study has been completed on the bleaching kinetics of visual pigments in intact retinas of the frog. The work confirmed the significant role of hydrogen ions as a co-factor with ATP in the formation of Metarhodopsin III. What is of particular interest is our finding that photoproduct decay is significantly dependent on the respiratory and metabolic environment of the receptors. Promise of substantial and important new ground to explore has been made evident by this instrumentation.

Personnel changes. Within the past 15 months seven scientists joined as full time working staff while five departed, returning to their permanent institutions. Many of our "visitors" have outside salary support so that the overall output is substantially larger than would otherwise be the case.

The principal NIH collaborations were with scientists in the NEI and NICHD. Collaborations also continue as in previous years with the University of Cambridge, England, the Italian Center for National Research in Pisa, the Australian National University and Duke University.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01659-09 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Synaptic Contacts of Retinal Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Lasansky	Research Biologist	LNP	NINCDS
OTHER:	S. Vallerga	Visiting Associate	LNP	NINCDS
	M. Hanani	Visiting Fellow	LNP	NINCDS

COOPERATING UNITS (if any)

None

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Section on Cell Biology

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Horizontal cells of salamander retina were intracellularly injected with horseradish peroxidase, and the histochemical reaction used to identify their processes and contacts at the level of the electron microscope. Horizontal cell bodies are linked to their axonal terminals by a very fine axon. Both segments make ribbon and distal junctions with rods and cones. Bipolar cells are postsynaptic to both axon terminals and cell bodies, but only the latter are in turn postsynaptic to bipolar cells. This bipolar cell input depolarizes the cell bodies when the surround is illuminated, and explains why their responses differ from those recorded at the axon terminals.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 01659-09 LNP

Project Description:

Objectives: To investigate the fine structure and function of synapses between retinal neurons.

Methods Employed: Electron microscopy combined with silver impregnations by the method of Golgi, and intracellular injections of horseradish peroxidase. Electrical recordings with intracellular microelectrodes.

Major Findings: A technique has been perfected for the intracellular injection of horseradish peroxidase through high-impedance microelectrodes. Salamander horizontal cells were stained by the subsequent histochemical reaction and found, as with earlier Procion yellow injections, to consist of two main parts, a cell body with dendrites and an axon terminal, from which different responses to light are recorded. In this instance, however, the electron opaque stain allowed the tracing with the electron microscope of the previously unidentified dendrites, which were seen to make ribbon and distal junctions with rods and cones, just as previously seen for the axon terminals. Furthermore, while bipolar cells are postsynaptic to both horizontal cell axon terminals and dendrites, only the latter are in turn postsynaptic to bipolar cells. This bipolar cell input depolarizes the horizontal cell bodies when the surround is illuminated, and explains why their responses differ from those recorded at the axon terminals.

Central illumination suppresses the depolarizing component of the horizontal cell body response as a consequence of the previously reported antagonistic effect of central illumination on the surround response of bipolar cells. Further analysis of this bipolar cell property revealed that central illumination only suppresses a fast component of the surround response, thought to be mediated by a new type of retinal neuron which is known, from as yet incomplete electron microscopical observations, to be postsynaptic to horizontal cell axon terminals and probably also to receptors, while presynaptic to bipolar cells.

Significance to Bio-medical Research and the Program of the Institute: It is hoped that these observations will help in identifying the mechanisms of synaptic transmission between photoreceptor cells and second order neurons, and provide a better knowledge of the neuronal networks involved in the processing of visual information within the retina.

Proposed Course of the Project: Electrophysiological work on salamander retina will be focused on a search for the unknown neuron thought to mediate the fast surround response of bipolar cells. Those cells exhibiting suitable response properties will be injected with Procion yellow and horseradish peroxidase to establish their location and pattern of branching, as well as whether they make the required synapses with bipolar cells, receptors and horizontal cells.

Publications:

Lasansky, A.: Synaptic organization of retinal receptors. In Barlow, H.B. and Fatt, P. (Eds.): Photoreception. London, Academic Press. In press.

Lasansky, A.: Interactions between horizontal cells of the salamander retina. Invest. Ophthal. 15: 909-916, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02221-02 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Ionic Mechanisms of Phototransduction in Rods of the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Normann	Staff Fellow	LNP NINCDS

COOPERATING UNITS (if any)
None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Membrane mechanisms by which light is transduced into electrical signals was investigated in rod type photoreceptors of the toad. The rod photoresponse results from two processes, a light modulated mechanism and a voltage and time dependent mechanism. Techniques for isolating each mechanism and the contribution of each to the photoresponse was studied.

Project No. Z01 NS 02221-02 LNP

Project Description:

Objectives: The principal objective of this study is to characterize the membrane mechanisms that are involved in the transduction of light stimulation into the electrical response of the photoreceptor.

Methods Employed: Intracellular recordings of electrical potential and conductance was performed in the isolated retina of Bufo Marinus using glass capillary microelectrodes. The retina was mounted in a chamber which allowed perfusion of the photoreceptors with solutions of various ionic compositions. Dissection of the preparation and microelectrode positioning was accomplished using infrared visualization provided with commercially available infrared to visible image converters.

Major Findings: The primary action of light stimulation on the electrical properties of rod type photoreceptors was investigated in the retina of the toad Bufo Marinus. All experiments were performed using an isolated superfused retina. Two membrane mechanisms are involved in the genesis of the rod photoresponse; a light modulated mechanism and a voltage and time dependent mechanism. When both mechanisms are stimulated by bright test flashes in either the eyecup preparation or the isolated retina, superfused with a physiological solution a normal response is seen which consists of an initial peak which decays into a sustained plateau.

Activation of the light modulated mechanism (which can be studied in isolation from the voltage and time dependent mechanism by increasing the potassium level in the superfusate to 10 mM) causes the rod to hyperpolarize up to a saturation potential. Further increases in light intensity only prolongs the response. The voltage dependent mechanism (which can be studied by passing extrinsic currents through the rod via the microelectrode) acts as a degenerative element in the rod membrane. Activation of the voltage dependent mechanism with either depolarizing or hyperpolarizing currents causes the membrane potential to sag back towards its prestimulus value. These two mechanisms combine to produce the rod photoresponse.

Significance to Bio-medical Research and the Program of the Institute: This project has significance primarily at the basic research level. Much is known about the ionic mechanisms involved in propagation of electrical signals along the nerve axons and in synaptic transmission. To date, no description of a primary sensory transducer has been presented which can account for the results described above. A comparison of the membrane mechanisms involved in the generation of the rod light response with the membrane properties of axons may provide fundamental insight into the basic mechanisms underlying neuron function.

Proposed Course of the Project: This project will be completed after a publication is completed and submitted.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02222-02 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Action of Calcium on Photoreceptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Bertrand	Guest Worker	LNP NINCDS
OTHER:	M. G. F. Fuortes	Chief	LNP NINCDS
	J. Pochobradsky	Visiting Fellow	LNP NINCDS

COOPERATING UNITS (if any)
None

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TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were made of the role of calcium ions in the transduction mechanism of turtle cone photoreceptors. It was concluded that EGTA, a calcium chelating agent, may open channels closed by calcium in darkness but differences in the response kinetics induced by light and calcium imply that calcium may not be the actual substance which initiates the light response.

Project No. Z01 NS 02222-02 LNP

Project Description:

Objectives: To investigate the role of calcium in the transduction process in the turtle photoreceptors.

Methods Employed: Cone responses were recorded intracellularly with glass microelectrodes in a perfused eyecup preparation. Internal or external concentration was varied, and the responses to various stimulus were recorded. A special perfusion system has been developed to change the external calcium concentration; the internal calcium concentration was varied by iontophoretic or pressure injection of a calcium chelating agent through the recording microelectrode.

To improve the speed and accuracy of data acquisition and storage, an on-line link-up with our PDP 11 computer system has been developed.

Major Findings: It was found that addition of EGTA to the external medium results in a depolarization of the membrane in the dark, a decrease of the amplitude of the responses to dim flashes, an enhancement of the amplitude and a slowing of the time course of the response to bright flashes. Low external calcium results in an increased membrane conductance, and this change can explain the depolarization of the cell in the dark and the modification of the amplitude of the response to both dim and bright flashes. Increased conductance, however, cannot explain the changes in the time course of the response. Lowering the internal calcium concentration results only in a decrease of the amplitude of the response. Experimental results are analyzed using the model developed by Baylor, Hodgkin and Lamb.

Significance to Bio-medical Research and the Program of the Institute: The purpose of this project is to advance our understanding of the excitation transduction process in photoreceptors.

Proposed Course of the Project: This project is terminated since the principal investigator returned to Switzerland.

Publications: A publication is in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02223-02 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Noise Analysis in Rods and Cones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Cervetto	Visiting Scientist	LNP NINCDS
OTHER:	F. Conti	Visiting Scientist	LNP NINCDS
	M. G. F. Fuortes	Chief, LNP	LNP NINCDS

COOPERATING UNITS (if any)
None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of calcium (Ca^{++}) depletion on the voltage characteristics of noise was measured and analyzed in turtle cone photoreceptors. The findings provide evidence that Ca^{++} does block light sensitive channels in the photoreceptor membrane but cannot prove Ca^{++} is the internal transmitter.

Project No. Z01 NS 02223-02 LNP

Project Description:

Objectives: To clarify the elementary mechanisms leading to photoreceptor responses.

Methods Employed: Turtle or toad retinae are isolated and perfused. Low-resistance microelectrodes are inserted in the retina and their noise (N_E) is measured. Retinal rods and/or cones are then impaled and the noise measurement is repeated first in light (N_L) then in darkness (N_D). Difference spectra are then constructed of N_D-N_L and N_L-N_E.

Major Findings: The amplitude of the voltage fluctuations (noise) across the membrane of turtle cones is close to maximal in darkness and is strongly reduced by bright illumination.

This finding is consistent with expectations based on a model system which imply that in darkness a considerable percentage of light sensitive channels through the membrane are already blocked and that release of transmitter following absorption of photons further reduces the number of conducting ionic channels.

Ca^{++} is believed to be the blocking transmitter responsible for the change in conductance in vertebrate photoreceptors.

Removal of extracellular Ca^{++} produces drastic changes in the noise characteristics of turtle cones: the voltage noise power is lowest in darkness, maximum for moderate light intensities, low again under bright illumination. The effect of Ca^{++} depletion on the voltage noise cannot be attributed to the concurrent suppression of synaptic inputs on cones because no effect is observed when synaptic transmission is blocked by cobalt in the presence of normal calcium.

These findings provide evidence that Ca^{++} does block light sensitive channels in the cones of turtle, but do not prove that Ca^{++} is the internal transmitter since it is not established yet whether blocking of channels occurs from intracellular side or from extracellular side or both.

Significance to Bio-medical Research and the Program of the Institute: Recent work has revealed new basic mechanisms of neuronal action. In photoreceptors, illumination evokes hyperpolarization and increased membrane resistance, suggesting that more "sodium channels" are open in darkness than during illumination. These results, together with the kinetic properties of the response led to the view that light causes the production of particles which block the sodium channels. Noise analysis should provide useful details on the mechanisms of this interesting action.

Project No. Z01 NS 02223-02 LNP

Proposed Course of the Project: Data is being analyzed and manuscripts for publication are in preparation.

Publications: None

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02230-02 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Computer Simulation of Photoreceptor Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Pochobradsky	Visiting Fellow	LNP NINCDS
OTHER:	M. G. F. Fuortes	Chief	LNP NINCDS
	D. Bertrand	Visiting Fellow	LNP NINCDS
	R. Normann	Staff Fellow	LNP NINCDS
	C. Wormington, III	Guest Worker	LNP NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on General Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Computer programs were developed to compare theoretical predictions with experimental data. The model of Baylor, Hodgkin and Lamb (BHL) was used to explain the effect of low and high Ca on turtle cone electrical responses to light. Ca hypothesis was tested on Bertrand's data. A modification of BHL model was proposed. Experiments of Normann with light adaptation and passing current were simulated with BHL model.

Project No. Z01 NS 02230-02 LNP

Project Description:

Objectives: a) Improved computer programs for photoreceptor models: To obtain solutions of BHL equations in a convenient form. Simulation is to be least-square fitted to experimental curves and graphically displayed. b) Ca hypothesis: To test its compatibility with Bertrand's results (project no. Z01 NS 02221-01 LNP), in the light of the BHL model. c) Generalization of the model of BHL: To explain the effects of low and high Ca on turtle cone responses, assuming Ca hypothesis. d) Light adaptation: To test whether Normann's experiments (project no. Z01 NS 02287-01 LNP) are explainable with the model of BHL. e) Future support of other projects: To provide mathematical programming assistance to other investigators, if needed.

Methods Employed: Reaction rate theory and models of compartmental systems. Numerical solution of differential and algebric equations by computer. Curve fitting with the MLAB program.

Major Findings: a) Programs supporting (b,c,d under objectives) have been developed. Some programs were given to the NIH Division of Computer Research and Technology for use in the new MLAB manual.

b) Experiments of Bertrand and Wormington with turtle cones in high Ca were designed to test Ca hypothesis. We concluded that they did not disprove it. The working hypothesis that Ca is the blocking substance is compatible with the observed changes of darkness potential, conductance and response kinetics. However, the model of BHL had to be modified.

c) Model of BHL was modified as follows: Some blocking particles are present even in darkness. What was the concentration of the precursor of the blocking substance is now the permeability of the OS membrane to Ca. What was the rate constant of creation of the blocking substance is now the concentration of extracellular Ca.

d) Responses of cones to pulses of current and changes in illumination have a shape predicted by the BHL model. Toad rod responses to pairs of flashes were explained on the basis of voltage and time dependent conductance. BHL model does not predict the observed decline of the "plateau" in response to steps of light.

Significance to Bio-medical Research and the Program of the Institute: Significance of the research of photoreceptor cells has been described in other reports of this laboratory. Our work deals with its quantitative aspects. Mathematical and theoretical analysis helps to explain experiments and to plan economically the new ones.

Project No. Z01 NS 02230-02 LNP

Proposed Course of the Project: a) completed. b)c) it is proposed to describe our findings in a short publication. A joint paper by Bertrand, Fuortes and Pochobradsky has been submitted for publication. d)e) We are ready to provide assistance in mathematical modeling and computer simulations to other investigators.

Publications: None

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02287-01 LNP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Light-adaptation in the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Normann	Staff Fellow	LNP NINCDS
OTHER:	I. Perlman	Visiting Fellow	LNP NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on General Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In response to a steady background the cone initially hyperpolarizes to a peak and then sags back to a potential level that is one-half the initial peak level. Just as in the dark-adapted state, the dynamic range of the light-adapted cone spanned about 3 1/2 log units but this range was shifted to encompass a brighter range of light intensities. When the photoresponses of the cones were compared to those of the horizontal cells under the same adaptation states it was observed that the cone to horizontal cell synapses produces additional effects. These effects caused the cone-horizontal cell system to have its highest gain for light modulations around the background level.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 02287-01 LNP

Project Description:

Objectives: To characterize the electrical properties of the turtle cone and horizontal cells under a variety of light-adaptation states produced by various levels of background illumination.

Methods Employed: Intracellular recordings of electrical potential generated by cones and horizontal cells were performed using glass capillary microelectrodes. The preparation studied was a turtle eyecup which was stimulated using a 2 channel photo-stimulator. Using this instrument test flashes of any intensity could be substituted for a wide variety of background illuminations.

Major Findings: The effects of background illumination on the electrical properties of cone type photoreceptors and horizontal cells was studied using intracellular recording techniques in the turtle retina. In the dark-adapted state, the cone produces graded hyperpolarizing responses to test flashes of graded intensities. The dynamic range of the cone is about 3 1/2 log units wide. When steady backgrounds are shone upon the retina, the cone initially hyperpolarizes but then sags back to a potential level that is 1/2 the initial peak level. After two minutes of equilibration of the cone to the background level, test flashes either brighter or dimmer than the background were substituted for the background. At any background level studied, the cone was able to generate graded responses. Just as in the dark-adapted state, the dynamic range of the light-adapted cone spanned about 3 1/2 log units but this range was shifted to encompass a brighter range of intensities.

Significance to Bio-medical Research and the Program of the Institute: The mechanism of homeostasis has been studied at a number of levels; whole organisms, organs and simpler neural networks. The study described herein has shown that a homeostatic mechanism is operating at the single cell level, adjusting its operating characteristics so that it can always respond to stimulation around any average value. Further, the measurements of sensitivity in the human visual system have shown that its dynamic range is also always about 3 1/2 log units and that this range is shifted by background illumination. This study has demonstrated that the origins of this phenomenon reside in the photoreceptors themselves.

Proposed Course of the Project: Two manuscripts are being prepared for journal submission. When these have been accepted by the journal, the project will be completed.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02288-01 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Role of Cyclic Nucleotides in Photoreceptor Sensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Wormington, III	Guest Worker	LNP NINCDS
OTHER:	H. G. Wagner	Acting Chief	LNP NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on General Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The phosphodiesterase inhibitor, 3-Isobutyl-1-methylxanthine, (MIX) was applied while recording intracellularly from single cones in a perfused eyecup preparation. This depolarized the cone membrane in the dark, decreased amplitude of responses to dim light, enhanced amplitude and slowed time to bright flashes. Results are being analyzed using the model of Baylor, Hodgkin and Lamb.

Project No. Z01 NS 02288-01 LNP

Project Description:

Objectives: To investigate the role of cyclic nucleotides in the transduction/adaptation processes in the turtle photoreceptor.

Methods Employed: Cone responses were recorded intracellularly with glass microelectrodes in a perfused eyecup preparation. Phosphodiesterase inhibitor was applied to the photoreceptors by superfusion. To improve speed and accuracy of data acquisition and storage, an on-line link up with our PDP 11 computer system has been developed.

Major Findings: It was found that addition of MIX to the external medium results in a depolarization of the membrane in the dark, a decrease of the amplitude of the responses to dim flashes, an enhancement of the amplitude and a slowing of the time course of the responses to bright flashes. Experimental results are analyzed using the model developed by Baylor, Hodgkin, and Lamb.

Significance to Bio-medical Research and the Program of the Institute: The purpose of this project is to advance our understanding of the excitation, transduction and adaptation processes in photoreceptors.

Proposed Course of the Project: Studies will be made to determine the role of cyclic nucleotides in the excitation/adaptation processes in photoreceptors. Signal averaging will be used to examine the effect of MIX on the time course of responses to dim flashes. Membrane conductance changes will be determined, and cyclic GMP and more membrane-permeant derivatives will be applied and their effects studied.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02290-01 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Measurement of Ionic Current from Photoreceptor Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. N. McBurney	Visiting Associate	LNP NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on General Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new technique is being developed which makes possible the recording of ionic current flow from the outer segment of single photoreceptors.

Preliminary results indicate that the technique is successful and that good recordings can be made of the responses to light in single photoreceptors.

Project No. Z01 NS 02290-01 LNP

Project Description:

Objectives: To develop a system capable of recording the ionic current flow in outer segment of single photoreceptors from toad retina.

Methods Employed: Under microscopic control the outer segment of single rods from slices of toad (Bufo marinus) retina is drawn into a tight fitting glass pipette. A current monitor connected between the inside of the pipette and the bathing solution records any current which flows across the membrane of the outer segments within the pipette. The current responses to light stimulation can then be investigated.

Major Findings: The initial period of this project has involved the development of the technique. There are no major findings as such, except that the technique is successful and that the measurement of current flow in single photoreceptors is possible. Preliminary experiments indicate that the responses to the capture of a single photon by a single photoreceptor can be measured.

Significance to Bio-medical Research and the Program of the Institute: These measurements are the first recordings of current flow from a single cell during a transduction process. It is hoped that a continuation of these experiments will add greatly to our knowledge of the processes underlying phototransduction.

Proposed Course of the Project: The technique which has been developed will be used to investigate the phototransduction process under a variety of stimulating conditions. A comparison will be made between current responses and intracellularly recorded voltage responses in toad rods in order to examine the effect of the cell's electrical properties on the photoresponses.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02291-01 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Study of the Role of Sodium and Potassium in the Cones of the Turtle Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Cervetto	Professor,	LINP CNR	Italy
OTHER:	M. G. F. Fuortes	Chief	LNP NINCDS	
	V. Torre	Professor	Univ. Genova	Italy

COOPERATING UNITS (if any)
Laboratory of Neurophysiology, CNR, Pisa, Italy

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on General Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed studies were begun of ionic properties of the membranes of cones of turtle. Special techniques were developed for perfusion so that rapid substitution of ions while recording the internal potentials of cones could be done.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 02291-01 LNP

Project Description:

Objectives: To understand more precisely the role of sodium and potassium ions in the transduction process in photoreceptors.

Methods Employed: Turtle retinas will be isolated and perfused. While recording intracellularly from receptors with very fine micropipette electrodes, membrane potential changes will be measured and correlated following rapid changes in the ionic strength of the perfusing fluid. These changes are then analyzed and interpreted with respect to other data and theoretical model photoreceptor systems.

Major Findings: The study is the stage of preliminary data collection.

Significance to Bio-medical Research and the Program of the Institute: This study should provide useful information on some of the elementary processes connected with the photo-excitation process. In particular it may be possible to establish directly whether the light responses are associated with a change in conductance of one or more ionic species.

Proposed Course of the Project: Data collection, analysis and reporting.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02152-03 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Neural connections in the retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Kolb	Senior Staff Fellow	LNP NINCDS
OTHER:	E. V. Famiglietti	Post-doctoral Fellow	LVR NEI

COOPERATING UNITS (if any)
National Eye Institute

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on Neuronal Interactions

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The inner plexiform layer (IPL) of cat retina can be subdivided into sublamina a, the 1/3 near the amacrine cells, and sublamina b, the 2/3 stretching to the ganglion cells, on the basis of differential branching of axons of bipolars and dendrites of ganglion and amacrine cells. The neurons making connections in sublamina a carry OFF center or darkness information and those making connections in sublamina b carry lightness or ON center information. Thus ON and OFF channels remain segregated and parallel in the retina through to the higher visual centers.

Project No. Z01 NS 02152-03 LNP

Project Description:

Objectives: To understand the neural circuitry of the vertebrate retina.

Methods Employed: 1. Light microscopy of Golgi-impregnated material. 2. Electron microscopy of Golgi-impregnated material. 3. Ultra-thin serial sectioning for electron microscopy. 4. Correlations with intracellular recordings and Procion marking of retinal neurons.

Major Findings: We have proposed on anatomical grounds that ON and OFF systems of the cat retina may be represented in separate sublaminae of the inner plexiform layer (IPL). These ganglion cells would be either ON center or OFF center depending on the level of the branching of their dendrites. This hypothesis has been tested in the perfused eyecup preparation of the cat retina by recording the light evoked responses of retinal cells intracellularly and injecting these cells with Procion or other fluorescent dyes. The results of 21 such injections and recordings has confirmed that ganglion cells (10) with dendrites stratifying in sublamina a, near the amacrine cell bodies give OFF center responses, whereas ganglion cells (10) stratifying in sublamina b closer to the ganglion cell bodies gave ON center responses. One ganglion cell proved to be an ON-OFF unit and its dendrites branched in both sublaminae a and b.

Four classes of ganglion cell (classes I, II, III and IV) can be distinguished on morphological criteria from Golgi preparations. Three of these classes consist of a (branching in sublamina a) and b (branching in sublamina b) pairs. By serial sectioning techniques for electron microscopy we have been able to analyze synaptic input to ganglion cells in the cat retina and we have determined that the parallel cone bipolar systems segregate their input to one of the a/b pairs of ganglion cells. Thus Ia, IIa and IIIa (ON center) ganglion cells receive input only from flat cone bipolars while ganglion cell types Ib, IIb and IIIb (OFF center) receive input solely from invaginating cone bipolars. Therefore, the ON and OFF systems of the cat may first arise at the level of these cone bipolars and their synaptic contact with the cones at the outer plexiform layer.

Significance to Bio-medical Research and the Program of the Institute: Studies of the structure of the retina will provide a reasonable understanding of the cells within the retina and will in all probability relate to neural circuitry elsewhere in the CNS. Many programs of this Institute are concerned with the physiology and marking of single retinal neurons and thus knowing the morphology and connectivity of these neurons is essential for our further understanding of visual events.

Proposed Course of the Project: It is proposed to continue the study of the structure of the retina to obtain further insights into the relevance of structure to function in the CNS.

Publications:

Kolb, H., Famiglietti, E.V. and Nelson, R.: Neural connections in the inner plexiform layer of the cat's retina. In Yamada and Mishima (Ed.): The Structure of the Eye III. Jap. J. Ophthal., Tokyo, Japan, 1976, 319-332.

Famiglietti, E.V. and Kolb, H.: Structural basis for ON- and OFF center responses in retinal ganglion cells. Science 194: 193-195, 1976.

Kolb, H. and Famiglietti, E.V.: Rod and cone pathways in the retina of the cat. Invest. Ophthal. 15: 935-946, 1976.

Nelson, R., Kolb, H., Famiglietti, E.V. and Gouras, P.: Neural responses in the rod and cone systems of the cat retina: Intracellular records and Procion stains. Invest. Ophthal. 15: 946-953, 1976.

Kolb, H.: The organization of the outer plexiform layer in the retina of the cat: Electron microscopic observations. J. Neurocytol. 6: 131-153, 1977.

Kolb, H. and West, R.: Synaptic connections of the interplexiform cell in the retina of the cat. J. Neurocytol. 6: 155-170, 1977.

Kolb, H. and Famiglietti, E.V.: Rod and cone pathways in the retina of the cat. Veterinary Digest In press.

Nelson, R., Famiglietti, E.V. and Kolb, H.: ON- and OFF-center ganglion cells in the retina of the cat: Intracellular staining. J. Neurophysiol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02289-01 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Functional Organization of the Visual Pathways in the Cat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. G. Wagner	Acting Chief	LNP NINCDS
OTHER:	W. Levick	Professor	ANU
	D. Kirk	Fellow	ANU

COOPERATING UNITS (if any)
Australian National University, Australia

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on Neuronal Interactions

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The lateral geniculate nucleus (LGN) of the cat was studied in terms of latency following electrical activation of the cortex and retinal afferents. These studies showed that the rarely encountered sluggishly responding classes relayed to the cortex as well as the brisk transient or sustained classes. The characteristics of LGN neurones compared to retinal afferents reinforce the idea that they are relay cells. Some brisk transient class cells from the contralateral eye have receptive fields which project well into the ipsilateral hemifield. Since these also relay to the cortex, presumably, each visual cortex is receiving information on the entire visual field.

ɔject Description:

Objectives: To study the various classes of retinal ganglion llɛ with respect to the projection of their receptive fields :o the lateral geniculate nucleus and cortex. This will provide ɔetter understanding of how a major sensory system organizes 1 transfers sensory information to the brain and in what form.

Methods Employed: Lightly anesthetized and curarized cats ə artifically respired and placed in a stereotoxic holder which rmits precise placement of electrodes in various parts of the sual system, in particular the retina, optic tract, lateral niculate nucleus and cortex. Manually placed targets are nipulated against a tangent screen background for visual imulation.

Extracellular recordings of physiological activity of various nglion cells in the retina and/or axones in the optic tract and rious sites in the lateral geniculate nucleus are analyzed with spect to changes in the visual display.

Major Findings: Lateral geniculate neurones of the cat were udied for latency of afferents and efferents by electrical mulation and by visual displays. Most of the units in the LGN re relay cells. A few were transsynaptically activated. Slowly nducting afferents from the retina were found which relayed to cortex. It was found that LGN cells with slow conducting ferents tended to have slow conducting axones and LGN cells with st conducting afferents seem to have fast conducting axones. relay cells tended to have very similar response properties that of the input retinal ganglion cell.

Brisk-transient LGN cells driven by axones from the contra- eral eye have receptive fields located up to 12° into the ilateral visual hemifield and were recorded in the region where er C merges with the medial interlaminar nucleus. These cells ry a direct projection of the contralateral temporal retina the visual cortex of a substantial part of the ipsilateral ual hemifield. In a few Siamese cats the decussation pattern retinal axones was studied by intraocular recordings coupled antidromic activation.

The proportion of crossed units in the temporal retina was ater and the region of intermingled units was much greater than the common cat.

The crossed-uncrossed projection from the retina did not have patchy variation expected on the basis of the alterations in laminar pattern of the lateral geniculate nucleus.

Significance to Bio-medical Research and the Program of the Institute: These studies help clarify the organization on information flow through the visual system to the brain. They elucidate principles and functional importance of the various pathways.

Proposed Course of the Project: This project will be completed as soon as manuscripts are prepared and published.

Publications:

Cleland, B.G., Levick, W.R., Morystyn, R. and Wagner, H.G.: Lateral geniculate relay of slowly conducting retinal afferents to cat visual cortex. *J. Physiol.* 255: 299-320, 1976.

INTRAMURAL RESEARCH PROJECT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Chloroquine Retinopathy in Rhesus Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Kolb	Senior Staff Fellow	LNP NINCDS
OTHER:	A. R. Rosenthal	Ophthalmologist	Stanford Univ.
	D. Bergsma	Senior Staff Ophthal.	CB NEI
	D. Huxsoll	Veterinarian	WRAIR

COOPERATING UNITS (if any)
National Eye Institute
Stanford University
Walter Reed Army Institute of Research

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on Neuronal Interactions

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The antimalarial drug chloroquine has been administered to Rhesus monkeys for a period of four years in order to evaluate its toxicity on the primate retina. Ophthalmological examination and electrophysiological evaluation of the monkeys showed no gross changes due to drug administration. Chemical analysis indicated accumulation of the drug in the pigmented ocular tissues while light and electron microscopy showed that pathological changes had occurred over the four year period.

Project No. Z01 NS 02292-01 LNP

roject Description:

Objectives: To understand the course of drug induced retinal
mage in a primate eye.

Methods Employed: Ophthalmoscopy, fluorocein angiography,
lectroretinography, chemical analysis, light and electron micro-
copy.

Major Findings: Rhesus monkeys have been administered high
ses (20mg/kg/day) of chloroquine hydrochloride for up to 4 years.
clinical picture of chloroquine retinopathy has not been achieved
any animal. Ophthalmoscopy, fluorescein angiography and elec-
oretinography remained normal for all monkeys, yet chemical
alysis of chloroquine content indicate massive accumulation of
e drug in the pigmented ocular tissues. Despite the normal
inical picture, the retinas of the experimental animals show
thological changes when examined by light and electron micro-
copy.

The first reaction of the retina to high doses of chloroquine
the appearance of membranous whorls (MCBs) in the neurons.
is pathology is reversible for the first year. At 1 year, how-
er, patchy photoreceptor and ganglion cell degeneration occurs
oceeding from perifoveal retina to involve the fovea at 3 years.
ter 4 years of high doses of chloroquine the central retina
ows extensive pathology of all neural layers: 50% of the
nglion cells are atrophied, large patches of photoreceptors show
generation and the pigment epithelium is folded and rounded off.
e fovea appears to be less affected. Low doses (7mg/kg/day)
weekly injections (20mg/kg/week) of chloroquine causes milder
t similar retinal changes.

Significance to Bio-medical Research and the Program of the
stitute: Antimalarial drugs are widely used in the treatment
the chronic collagenoses in man but unfortunately have a major
de effect on the eye often causing blindness. Thus studying
loroquine induced damage to the Rhesus monkey retina allows us
me understanding of the earliest changes in a retinal degenera-
on in an animal model close to man. The pathological changes
at occur in the retina with chloroquine resemble changes seen
Tay-Sachs Disease and Sphingolipidoses which should be of
terest to people studying such diseases.

Proposed Course of the Project: We intend to analyze and
nsolidate our findings for publication.

blications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02293-01 LNP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neural Integration and Processing in the Mammalian Visual System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. G. Wagner	Acting Chief	LNP NINCDS
OTHER:	M. L. Wolbarsht	Professor	Duke Univ.
	R. Crocker	Pre-doctoral Candidate	Duke Univ.
	J. Ringo	Pre-doctoral Candidate	Duke Univ.

COOPERATING UNITS (if any)

Duke University
Durham, N.C.

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on Neuronal Interactions

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.35	0.25	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study of the spectral sensitivity of retinal ganglion cells in the cat has been made using strong chromatic adaptation and spatial localization of the stimulus to the receptive center and/or periphery. The results have shown that three independent and chromatically distinct cone receptor systems appear to converge on many if not all ganglion cells in the retinas of cats. Most show simple additivity rather than opponency in the same region of the receptive field. A rare cell did show opponency however.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 02293-01 LNP

Project Description:

Objectives: To study neural interactions and processing in a major sensory system. This study will provide insight on how information is organized and processed by a major neural plexus (retina) in preparation for transmittal to a distant neural plexus (lateral geniculate) nucleus.

Methods Employed: Anesthetized and curarized intact experimental mammals such as the cat and monkey are placed in a special holder for stereotoxic placement of a interocular microelectrode to the retina. A modified maxwellian view optical stimulator permits precise light stimuli to be placed on the retina under direct visualization. Electrical responses are induced by light which are correlated with various parameters of the stimulus.

Major Findings: Cat retinal ganglion cell types X, Y and W show three independent input systems believed to be cone systems which can be differentiated on the basis of spectral sensitivities. One, previously shown by others, has its maximum sensitivity (Lambda max) at 450 nm. Another also well known, has its λ max at 550. The third one has been confused with the rod system because it has the same λ max of 500 nm.

Confidence in our interpretation that it is in reality a cone system is based on receptive field differences, cone to rod break during dark-adaptation and that the determinations were made in the presence of background light levels well into photopic levels.

Significance to Bio-medical Research and the Program of the Institute: This study suggests that the cat as well as other mammals have trichromacy in their retina's even though not well expressed in their behavior. The presence of three separate wavelength independent systems may be more broadly represented in the animal kingdom.

Proposed Course of the Project: Further study will be made on color coding characteristics of the receptive fields and ganglion cell classes in cats and monkeys.

Publications:

Ringo, J., Wolbarsht, M.L., Wagner, H.G., Crocker, R. and Amthor, F.: Trichromatic vision in the cat. Science In press.

INTRAMURAL RESEARCH PROJECT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Rapid Scanning Microspectrophotometry in Visual Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. G. Smith, Jr.	Medical Officer	LNP	NINCDS
OTHER:	J. Oldak	Staff Fellow	LNP	NINCDS
	T. Colburn	Electronic Engineer	TD	NINCDS

COOPERATING UNITS (if any)

Dept. of Biomedical Engineering
University of Maryland

Section on Technical Development
NIMH, NINCDS

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on Sensory Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A rapid scanning microspectrophotometer has been designed and constructed which allows absorption spectra to be measured between 3 and 650 nm at a speed of 0.6 msec per wavelength band (10 nm). Experiments on frog (Rana pipens) have been performed or are in progress to measure the effects of several variables on visual pigment (rhodopsin) kinetic changes. These variables include pH, availability of glucose and oxygen, temperature, divalent cations presence and absence of pigment epithelium and degree of attachme of rod outer segments. The results have confirmed the importance of pH in determining the rhodopsin photoproduct pathways and kinetics. For the first time, however, the metabolic and respira status of the retina can be paramount in determining photoproduct characteristics.

PHS-6040
(Rev. 10-76)

Project No. Z01 NS 01690-09 LNP

Project Description:

Objectives: The objective of this research is the development of rapid scanning microspectrophotometric techniques for investigation of the chemical kinetics of excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cell physiology will also be considered.

Methods Employed: The microspectrophotometer sequentially samples transmittance at spectral wave bands between 350 and 650 nm, at rates of 600 microseconds per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by the image of a cathode ray tube face.

Major Findings: The first complete set of experiments have been completed. These experiments varied, in a systematic way, the effects of pH, metabolism and respiration on the pathways and kinetics on the rhodopsin photoproducts, metarhodopsins II and III and retinal. The pH was set at 5.5 with acetate buffer, 7.4 with TRIS and 8.2 with HEPES. Metabolism was controlled by adding glucose to the perfusate to stimulate metabolism or 2-deoxy-glucose to block metabolism. Respiration was controlled by saturating the perfusate with oxygen or nitrogen to stimulate or block respiration, respectively.

At low pH (5.5), pH was found to be the dominant factor in controlling photoproduct characteristics, whereas at pH 7.4 and especially at pH 8.2, metabolic and/or respiratory were sometimes more important.

Significance to Bio-medical Research and the Program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need has developed.

Prior to the completion of these experiments there was considerable discussion, confusion and disagreement among scientists as to what were "the true" pathways and kinetics of the photoproducts of the visual pigment rhodopsin. What these experiments have shown, for the first time, is that there is not a single set of photoproduct characteristics, but that the pathways and kinetics depend on the experimental situation. In retrospect, it appears that the varied results reported by previous investigations probably reflects differences in experimental conditions rather than "correct" or "incorrect" results. Cleaning up this confusing situation should help set photopigments and photophysiological research along a more productive course of endeavor.

Of potential importance to central nervous system research is our paradoxical finding that the fucntional consequences of anoxia are less severe if glucose metabolism has been reduced (with 2-deoxy-glucose) than if functioning normally (with glucose). A similar "paradoxical" result has been reported from CNS studies, however, it has been difficult to document precisely because of the absence of easily quantifiable parameters of brain function in those experiments. Thus, the retina, a CNS-appendage, where precise, quantitative measures of biologically important functional parameters can be obtained, may prove a useful preparation for assaying CNS function in various metabolic, respiratory and other states.

Our research may also be of relevance to an understanding of the pathophysiology of retinal diseased states. Heretofore, most retinal signs and symptoms were held to be mainly neural in origin. Our demonstration that abnormal retinal conditions can lead to visual pigment and hence to photoreceptor malfunction.

Proposed Course of the Project: The studies on the photoproduct pathways and kinetics will be expanded in two ways. First, the capabilities of the machine will be improved, in terms of its speed of performance, so that the characteristics of an earlier photoproduct, metarhodopsin I can be studied. A knowledge of metarhodopsin I's properties is important because of its candidacy as the photoproduct which initiates photoreceptor electrical events.

Second, the role of other parameters, like temperature and divalent cations, in determining photoproduct pathways and kinetics will be performed.

Publications: None

INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Electrophysiology and Neuropharmacology of Simple Cellular Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Barker	Medical Officer	LNP NINCDS
	T. G. Smith, Jr.	Medical Officer	LNP NINCDS
OTHER:	K. Futamachi	Staff Fellow	LNP NINCDS
	D. Gruol	Guest Worker	LNP NINCDS
	P. G. Nelson	Chief	BB NICHD
	W. A. Klee	Research Scientist	LCB NIMH
	R. Macdonald	Guest Worker	BB NICHD
	J. Neale	Guest Worker	Georgetown Univ.
	W. Sheriff	Computer Specialist	TD NINCDS
	W. Stewart	Research Associate	LEP NIAMDD

COOPERATING UNITS (if any)

Behavioral Biology Branch, NICHD
Sect. on Technical Development, NINCDS
Lab. of General and Comparative Biochemistry, NIMH
Dept. Biochemistry, Georgetown University
Lab. Experimental Pathology, NIAMDD

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on Sensory Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.7	4.6	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electrophysiological experiments with microelectrodes have been performed on mouse central nervous system neurons grown in tissue culture and on identified molluscan central neurons. Intracellular recording techniques, including voltag clamp analysis, have been used to study the physiology and pharmacology of these cells. Vertebrate peptides have been found which directly and significantly alt the basis of excitability of specific molluscan neurons, leading us to propose them as neurohormones. Research on tissue-cultured neurons has been directed at the mechanisms of action of clinically important anticonvulsants, anesthetics and convulsants. The tissue culture preparation has allowed us to examine the amino acid neuropharmacology of central neurons in much finer detail than had pr iously been possible. Almost all of the clinically relevant drugs appear to be modulating the pharmacology of the inhibitory amino acid GABA and the excitatory amino acid glutamate. Those that induce convulsions depress GABA pharmacology and those that reduce convulsions or anesthetize enhance GABA and/or depress glutamate pharmacology. The results suggest that tissue-cultured mouse central neurons are a useful model for high resolution study of CNS neurophysiology and neuropharmacology.

ect Description:

Objectives: The objective of this research is to gain in-
t into how membrane mechanisms and intercellular connections
responsible for the electrical activity of single cells.

Methods Employed: The electrical properties of single cells
recorded with intracellular microelectrodes and analyzed
r a variety of experimental conditions. The technique of
age-clamping the membrane potential is the most important
od employed in these experiments. This technique requires
penetration of single cells with two microelectrodes, one
monitoring the membrane potential, the other for supplying
current necessary to clamp the membrane potential at the
red level. Using this technique the role of the various
c conductances responsible for the electrical responses of
membrane can be studied. In addition, the effects of chang-
the intracellular and/or extracellular ions and of adding
macological agents on membrane properties can be evaluated.

Because of the necessity of taking many measurements from
experiment, the data is often recorded on magnetic tape for
r analysis with a PDP-11 computer.

The anatomical properties of the cells are studied with
entional light and electron microscope techniques.

Major Findings: 1. Tissue-cultured nerve cells. Nerve
s derived from embryonic mouse spinal cord and grown in
ue culture manifest many of the properties characteristic
pinal neurons studied *in vivo*: electrical excitability,
ptic activity, and chemosensitivity. Since the cells show a
uitous sensitivity to amino acids, we have focused first on
ining the details of their amino acid pharmacology. The
ral amino acids γ-aminobutyric acid, β-alanine and glycine
depress excitability by increasing membrane conductance to
ride ions. Glutamate excites all neurons tested, probably
ncreasing membrane conductance to sodium ions. Individual
ons show a topographic anatomical distribution of chemo-
itivity to the putative amino acid transmitters with excita-
responses to the neutral amino acids present on some, but
all anatomical processes. Analysis of the dose-response
es indicates that probably two GABA molecules but only one
amate molecule participates in the unitary post-synaptic con-
ance change. The GABA response thus varies in a non-linear
er with GABA concentration, while the glutamate response
es in a linear way.

The effects of the convulsants picrotoxin, pentylenetetra-
, penicillin and bicuculline on the physiology and pharma-

cology of spinal neurons have been studied. All four agents duced paroxysmal depolarizing shifts in the spontaneous acti of the cells, indicating that the CNS in tissue culture has served and maintained the substrate necessary for convulsive electrical activity. All four agents selectively reduced GA responses without affecting those to other amino acids. Lit if any direct effects on neuronal membrane properties were observed. The results suggest that the selective attenuatio GABA-mediated post-synaptic inhibition may help account for convulsant properties of the four agents.

The effects of anti-convulsant and anesthetic barbitura on the physiology and pharmacology of spinal neurons have al been examined. The anesthetic, but not the anti-convulsant of barbiturate, directly depressed neuronal excitability, ap ently by activating GABA receptors. When the anesthetic bar urates were bath applied, all cells became electrically sile though not inexcitable. The anti-convulsant barbiturates di alter spontaneous neuronal activity but greatly attenuated epileptic episodes induced by one of the convulsant agents. classes of barbiturate enhanced GABA inhibitory responses an depressed glutamate excitatory responses in a dose-dependent versible manner without affecting responses to β-alanine or glycine. The anesthetics were considerably more potent than anti-convulsants. In addition, the anesthetic but not the a convulsant agents prolonged the time courses of the GABA responses. The results suggest mechanisms of action for two structurally similar classes of clinically important drugs a membrane level. Barbiturate anesthetics may anesthetize by directly activating GABA receptors and by potently enhancing inhibition and depressing glutamate excitation. The anti-convulsant barbiturates reduce hyper-excitability by enhanci GABA inhibition and reducing glutamate excitation in a less potent manner.

Recently, we have successfully voltage-clamped tissue culture spinal cord neurons and recorded the membrane curren evoked by iontophoresed amino acids. In addition, we have f that one opiate pentapeptide, leucine-enkephaline, can modul the effects of the amino acids on spinal neurons. Such expe ments should allow a more precise analysis and characterizat of neurotransmitter and neurohormonal mechanisms.

2. Molluscan neurosecretory cells.

Using the voltage clamp technique we have been able to identify the conductances associated with endogenous pacemak activity and the conductance underlying the change in spike perties during the burst of spikes evoked by the pacemaker activity. The pacemaker activity is due to a sequential act vation of a voltage-dependent Na^+ conductance and then a voltage-dependent K^+ conductance. Both "pacemaker conductances" are regulated by Ca^{++} ions.

The kinetics of inactivation of the K^+ pacemaker conductance control the periodicity of the pacemaker activity while the Na^+ pacemaker conductance provides the depolarizing impetus for evoking a burst of spikes at the depolarizing crest of each pacemaker potential. The progressive prolongation of successive spikes during the burst is due to a frequency-sensitive spike K^+ conductance (delayed rectification) which repolarizes spikes. The faster the conductance is used, the more it inactivates, thus providing less repolarizing force. Hence, the spikes become prolonged.

Two vertebrate neuronal peptides - lysine vasopressin (LVP) and oxytocin - altered the excitability of identified snail neurons in a way quite distinct from the actions of conventional neurotransmitters. One effect of these peptides is excitatory, characterized by the induction or enhancement of bursting pacemaker potential (BPP) activity in two specific cells in Otala and Aplysia. The BPP activity outlasts the period of peptide application by hours with a threshold concentration at the nanomolar level. In contrast, putative neurotransmitters applied to the same cells only transiently alter excitability at micromolar concentrations. Similar long-lasting enhancement of BPP activity has been reported following stimulation of a remote cluster of neurosecretory cells (Bag Cells) which do not make synaptic contact with the peptide-sensitive cell. Peptide extracts of the Bag Cells produce effects similar to Bag Cell stimulation and to LVP application. These results suggest that the Bag Cells synthesize and release a peptide whose structure overlaps LVP. The Bag Cell peptide then diffuses through the hemolymph to act on target nerve cells like a neurohormone. We have used the more readily available LVP, rather than Bag Cell extract, in conjunction with voltage clamp technique, to investigate the biophysical mechanisms underlying the peptide effects. Much of the work has been done with the peptide-sensitive cell in the land snail since the cell does not generate BPP activity when the snail is dormant and thus provides better baseline reference for examination of LVP effects.

Voltage clamp analysis shows the excitatory effects of the peptide are due primarily to activation of the Na^+ and K^+ pacemaker conductances. Additionally, there is a shortening in the decay time of the K^+ pacemaker conductance by the peptide.

What are the consequences of peptide-induced BPP activity? Spikes generated during the depolarizing phase of the BPP become progressively longer in duration and their after-potentials become increasingly shortened. These self-excitatory mechani are themselves not altered by LVP, but rather are activated b the underlying BPP activity induced by the peptide. The progressively longer spikes evoked during a BPP are correlated w progressively greater entry of Ca^{++} into the cell body. If t burst of spikes is faithfully propagated along the cell's axo and invades its terminals, and if similar frequency-sensitive spike-related K^{+} conductance mechanisms exist there, then a facilitated entry of Ca^{++} should likewise occur, and lead to pulsatile, facilitated release of neurotransmitters of neurosecretory product. Peptide-induced BPP activity thus should vide for a pulsatile, facilitated release. Additionally, the introduction of a non-linear membrane by the peptide should transform the input-output relations of the cell, so that les synaptic input has considerably more effect on output.

An unusual inhibitory effect of LVP on another molluscan neuron has been observed on several occasions. The effect is characterized by hyperpolarization of the cell with an increa in membrane conductance, rendering the steady-state I-V curve nearly linear with a positive slope. Spikes generated in the presence of LVP are smaller in amplitude. All of these effec are reversible within minutes by renewal of the peptide or by changing Sr^{++} for Ca^{++} in the medium. The reduction in spike in this case appears to be a direct effect of LVP on the vol dependent conductance underlying the spike. The results of t studies suggest a new form of chemical communication between neurons which is extrasynaptic, mediated by peptides, and cha terized by regulation of the excitability of specific target cells, which have the appropriate membrane receptors for the peptides, through effects on voltage-dependent and voltage-independent conductances. (Neurotransmitters, by contrast, a ently alter excitability exclusively by regulating voltage-independent conductances.) Thus, peptides act as "neurohormo by modulating the cell's excitability to synaptic inputs. Si forms of neurohormonal communication may exist in the vertebr

3. Other Molluscan neurons

As reported last year Dr. Futamachi discovered that cert experimental maneuvers cause otherwise normal cells to produc a variety of electrical activity qualitatively similar to tha found in vertebrate epileptogenic neurons namely paroxysmal d polarizing shifts and bursts of spikes. These maneuvers incl alteration of the divalent cations in the extracellular fluid Dr. Futamachi found that the epileptiform activity is correla with changes both in the conductances of the membranes of the affected cells and in the interconnections between those cell In addition, he has found that the paroxysmal bursts occurred synchronously in a population of cells. In an effort to be able to locate pairs of synchronous cells most reliably Dr. Futamachi used a new fluorescent dye, developed by Walter Stewart, for marking the cells studied. This dye is electrically injected through an intracellular micropipette and stains all the processer of the neuron within a matter of minutes, without apparently altering the electrical properties of the neuron.

Recently, Dr. Futamachi has begun a study of the mode of action of pharmacological agents on this epileptogenic-like activity. These agents include both those known to cause or enhance epilepsy as well as those known to be of therapeutic benefit in clinical and experimental conditions.

Significance to Biomedical Research and the Program of the Institute: The tissue-cultured mammalian central nervous sytem appears to offer an extremely useful preparation for studying the basic physiology and pharmacology of central neurons. The electrophysiologic mechanisms of action of various clinically important neuropharmacologic drugs can be studied with relative ease and increasing rigor. Thus far we have investigated a variety of convulsants, anti-convulsants and anesthetics and have been able to provide a phenomenology of pharmacologic effect not previously observed. We plan to continue study of receptor pharmacology extending the research to other drugs and other receptors and to both chronic as well as acute application. In addition, we have begun to investigate the role of peptides in neuronal function, drawing upon the results with the Molluscan systems as a useful reference.

Central to understanding communication in the central nervous system is an understanding of the physiology and pharmacology of its individual elements. Our research has focused on receptor pharmacology since this aspect of cell-to-cell communication is relatively easily studied and yet plays a crucial role in intracellular communication. We have been able to resolve details regarding amino acid receptors not previously appreciated. It is also clear from our initial studies that a variety of clinically important drugs affect receptor-coupled changes in excitability and that these changes may well be the basis for their pharmacologic effects in the CNS. The results may thus help to raise clinical neuropharmacology from its empirical realm to a more solid scientific basis.

Proposed Course: The main thrust of this project's future research will employ tissue-cultured vertebrate nerve and muscle cells to analyze the membrane mechanisms underlying the electrical activity of such cells. Molluscan studies will continue to the extent that they can supplement the tissue culture work.

The synaptic neuropharmacology will proceed, with greate use of the voltage-clamp technique. Such experiments should provide more meaningful and quantitative analyses and, hopefu a better understanding of the membrane mechanisms underlying neurotransmitter action. Most of these experiments have beer performed on mouse spinal cord neurons. In the future, we pl to investigate more supraspinal neurons.

In collaboration with Drs. Joe Neale and Werner Klee, we are in the process of analyzing mammalian CNS nerve cells for opiate peptide receptors. The initial aim of this research i to be able to determine if tissue-cultured CNS has opiate peptide receptors and further, which cells possess these part lar peptide-receptors. The main aim of this work will be an attempt: 1) to identify the electrophysiological events whic result from the application of opiate and other peptides to tissue cultured neurons and 2) to determine the membrane mechanisms underlying such events. Central questions, in thi regard, are do peptides act on membrane conductances, particularly voltage- and time-dependent ones, like in molluscs or they act more like neurotransmitters? Finally, we plan to lo those neurons which synthesize particular peptides using immu histochemical techniques.

Both in tissue cultured vertebrate neurons and in mollus neurons, an investigation will continue on the mode of action of known convulsants and anti-convulsants at the single cell level. Thus far our experiments have shown that such agents have effects both on the non-synaptic membrane and on synapti transmission. Clearly, these agents can have several modes o action and our effects will be directed to defining them more precisely and to assessing their relative importance.

Publications:

Ransom, B.R. and Barker, J.L.: Pentobarbital selectively enhances GABA-mediated post-synaptic inhibition in tissue-cultured mouse spinal neurons. Brain Res. 114: 530-535,

Barker, J.L., Neale, J.H. and Bonner, W.M.: Slab gel analy of rapidly transported proteins in the isolated frog nervou system. Brain Res. 124: 191-196, 1977.

Barker, J.L.: Physiological roles of peptides in the nervous system. In Gainer, H. (Ed.): Peptides in Neurobiology. New York, Raven Press, 1977, pp. 295-343.

Barker, J.L.: Peptide regulation of neuronal excitability: evidence for a neurohormonal role. In Buckly, J.P. (Ed.): International Symposium on the Central Effects of Angiotensin and Related Hormones. New York, Pergamon Press. In press.

Macdonald, R.L. and Barker, J.L.: Pentylenetetrazole and penicillin are GABA-selective antagonists on cultured mammalian neurons. Nature. In press.

Barker, J.L. and Smith, T.G.: Peptides as neurohormones. In Cowan, W.M. and Ferendelli, J.A. (Eds.), Neuroscience Symposia, Volume II. Approaches to the Cell Biology of Neurons. London, Macmillian Press. In press.

Barker, J.L. and Smith, T.G.: Peptides roles in neuronal function. In Suria, A. and Costa, E. (Eds.), Textbook of Neuropharmacology. New York, Raven Press. In press.

Annual Report
July 1, 1976 thru Sept 30, 1977
National Institute of Neurological Communicative
Disorders and Stroke
Laboratory of Biophysics
William J. Adelman, Jr., PhD, Chief

INTRODUCTION

The research program of the Laboratory of Biophysics is concerned with investigating molecular and cellular mechanisms responsible for excitation, membrane potentials, the generation of the nerve impulse, and synaptic activity. This program involves the study of natural membranes and artificial membrane systems. The laboratory is concerned with the biophysical basis for the functioning of simple nervous systems and with uncovering the cellular basis for such integrative neural functions as behavior and learning. The laboratory makes wide use of physical and chemical techniques, on-line and off-line digital computers and a variety of applied mathematical methods. The laboratory is composed of two units. One of these units operates on a year-round basis at the Marine Biological Laboratory in Woods Hole, Mass. The Woods Hole Unit is composed of 2 sections; the Section on Neural Membranes and the Section on Neural Systems. The Bethesda unit of the laboratory is made up of the Section on Molecular Biophysics. This report covers the second year of operation of the Woods Hole Unit of LB. The advantages of having a unit at the MBL are now clear: 1) It has now been unequivocally demonstrated that squid giant axon experiments can be run from the end of April through the end of November. In 1976 two major squid axon projects were completed in the fall after the traditional summer squid season ended. 2) Efforts at cultivating marine nudibranchs have now resulted in a number of second generation cultured animals. The superb marine environment at the MBL has allowed this program to reach this level of success. 3) Studies on the behavior of *Hermissenda* crassicornis have reached a stage where long-term learning in these animals has been demonstrated. 4) The Woods Hole unit continues to provide a unique intellectual and physical environment for attracting both postdoctorals and senior collaborative scientists from a variety of Universities and from the NIH.

WOODS HOLE UNIT OF THE LABORATORY OF BIOPHYSICS:

Section on Neural Membranes.

The Section on Neural Membranes has been concerned with characterizing, in as much detail as possible, the model nerve fiber formed by the squid giant axon. Intracellular, membrane, and extracellular components of this total system are studied. The primary thrust has been the examination of sodium and potassium channels in the axon membrane. These studies have been concerned with a variety of ionic interactions within these channels and with the effects of divalent ions on the kinetics of such phenomena as sodium inactivation.

Extensive studies are being carried out on the makeup of the intracellular neural cytoskeleton and its protein structure. These studies have centered on characterizing both the protein makeup and the ultrastructural characteristics of axoplasmic neural filaments. In addition, the section has continued to probe the role of the Schwann sheath in axonal functions. Studies on muscle mechanics and on mechanotransduction in nerve were planned and are now underway.

The experimental program of the Section has made the following advances. Studies on ionic interactions and selectivity in the K channel have continued with an examination of the effects of various ions in internally perfused axons. Methylammonium and tris (hydroxymethyl) aminomethane were shown to produce a voltage dependent block of K currents. Some data were obtained using internal rubidium and ammonium, and more detailed studies were carried out using internal sodium and cesium. An interesting result of the sodium studies is that the K channel begins to show a significant permeability to sodium at voltages $\geq$ 150 mV (inside minus outside). Future experiments are planned to further investigate kinetics and the effects of temperature on the ionic interactions. As part of the ion-interaction projects a study of the effects of external calcium upon sodium inactivation in squid axon was undertaken. The effect of varying concentrations of calcium upon sodium inactivation was studied with a prepulse-test pulse voltage clamp regime. The effect of calcium was found to be consistent with a charge screening theory for steady state inactivation behavior, but kinetic effects were in strong disagreement with this theory. Calcium and magnesium do not behave as equimolar substitutes which is a new finding in disagreement with work on other preparations.

The collaborative effort with Carnegie-Mellon University on squid and *Myxicola* axon neural filaments has been successful in isolating and concentrating neural filaments of 10 nm diameter from the axoplasm of *Loligo pealei*. The major proteins in the concentrated suspensions included a 200,000 dalton protein which comigrated with rabbit myosin, and proteins with molecular weights of 160,000 and 63,000. Isolated filaments have been concentrated by filtration and these have been examined by electronmicroscopy and by biochemical methods. The Section has continued its studies on the Schwann sheaths enclosing the giant axons of both squid and *Myxicola*. On the basis of montage electronmicrographs, it has been possible to derive values for volumetric size of the Schwann cell layer extracellular space and the capacity of the Schwann cell membrane. A paper based on a model reconstruction of the Schwann cell layer has been submitted and accepted for publication. Analysis of impedance measurements made on the squid giant axon continue to indicate that these are consistent with the anatomy of the Schwann sheath as revealed by electronmicroscopy.

Another project has studied the effects of unstirred layers near ion exchange membranes on transmembrane potentials. Using numerical methods and appropriate analytic approximations, the Nernst-Planck equations were solved for the transmembrane voltage across an ion exchange membrane. The calculations defined conditions under which the dominating Donnan component of the total potential difference is extremely sensitive to the thickness of unstirred solution layers at the membrane boundaries. Data from the literature was shown to be consistent with the approach used.

In a new project related to photo-transduction, the technique of microspectrophotometry was utilized to study the light absorption properties of visual pigments. In the course of investigating the distribution of the types of vertebrate visual pigments, several species of *Percids and Cichlids* were investigated. In an attempt to correlate structure and function in the retina, one of these fishes was studied in detail, yielding the following new results: 1) *Nannacara anomala* retinae have only 3 types of photoreceptors: rods, single cones and double cones. The cones are arranged in mosaics: a central single cone is surrounded by 4 double cones in a regular square pattern. The single cones are found to contain only a blue-absorbing pigment, the double cones, two slightly different pigments: one a yellow-absorbing and the other an orange-absorbing. The rods contain a distinctly different pigment, most sensitive to green light. The four visual pigments are probably based on vitamin A_2.

One of the major projects and services rendered by the Laboratory of Biophysics has been the development of data acquisitions systems for obtaining excitable membrane conductance data in a form that can be analyzed by digital computing machine. The latest advance in this area was the improvement in Woods Hole of a data acquisition system adding to the PDP-11/10 digital computer system additional floppy disks, a second 9-tract read-write magnetic tape and an electrostatic line-printer as peripherals. This system is now being used in the study of ion interactions in sodium and potassium channels in the squid giant axon by the Section on Neural Membranes. The general configuration of this unit and its software operating system has been used as a model by the Section on Technical Development of NINCDS/NIMH for designing and constructing systems to be used by other laboratories in the Institutes. New techniques for measuring impedance of giant axon membranes with the voltage clamp involving small signals have been developed. In addition a system has been devised for measuring current noise from membrane channels.

The above highlights of the program of the Section on Neural Membranes are examples of an approach to understanding the excitable membrane at the cellular, subcellular and molecular levels.

The Editorial Committee of Annual Reviews of Physiology invited a senior member of the Section to write the Prefactory Chapter for Vol. 41. These chapters are "traditionally authored by a physiologist of great distinction". The same senior member was also made Honorary Chairman for a N. Y. Academy of Science Conference on "Electrical Properties of Biological Polymers, Water & Membranes" on January 26-28, 1977, and at the Annual General Meeting of the Physiological Society (London) March 26, 1977, was elected an Honorary Member after he had consented to have his name placed in nomination.

At the Annual Meeting of the Society of General Physiologists, Sep 1976, the Laboratory Chief was elected Treasurer of the Society, and assumed office January 1, 1977.

Section on Neural Systems.

The principal objective of the section is to study the mechanisms by which simple neural networks process information with particular emphasis on cellular mechanisms of learning. The nervous system of *Hermissenda crassicornis* has proven to be an excellent model for information processing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in synaptic circuitry produced by conditioning paradigms administered to intact animals as well as to isolated nervous systems, membrane properties modified by conditioning, identification of critical developmental stages for the neural networks studied as well as stages critical for learning.

Although in the past many neural preparations have been studied with similar goals, none have been understood with the precision necessary to ask questions about neural network function on a cellular level. Because a cell by cell analysis has been possible for the visual and vestibular pathways of *Hermissenda,* cellular principles of network function have emerged. Presynaptic inhibition, for example, has now been shown to be responsible for persistant changes of network function. This cell by cell analysis has in the past been made by multiple simultaneous intracellular recording and has now been extended to include electronmicroscopic identification of synapses.

The approach of the Section, because of its residence at the Marine Biological Laboratory, can be broad both in the scientific methods employed as well as in its spectrum of biological interests. A large number of marine forms at different evolutionary stages, are available for comparative studies. The neural organization of the eye of *Elysia*, a nudibranch indigenous to Cape Cod, for instance, has been the subject of histologic, behavioral and electrophyiologic analyses. This nudibranch has also been cultured in our laboratory. Flowing seawater holding facilities make possible long-term behavioral and correlated electrophysiological and biochemical analyses. Several features of vertebrate learning, for instance, have just recently been identified for associate training of *Hermissenda*. These findings suggest that the cellular mechanisms uncovered in the less evolved species of *Hermissenda* could eventually have clinical significance. Mariculture of successive *Hermissenda* generations has also just recently become a reality.

Among the techniques employed thus far are the following: simultaneous intracellular recording from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophoresis of fluorescent dyes and electron dense materials, automated behavioral monitoring, subcellular fractionation, protein phosphorylation analysis and uptake of neurotransmitter precursors, in vitro mariculture of nudibranch species.

Taking a broad approach in pursuit of its primary objective, the section has brought together a number of scientists highly trained in a variety of disciplines: electrophysiology, animal behavior, biochemistry, histology and embryology. This breadth of scientific technique and biological perspective are already yielding important results. These include cellular mechanisms of conditioning, nonsynaptic interneuronal communication, transducer properties of hair cells, electronmicroscopical identification of synaptic endings, neurochemistry of network elements, and veliger physiology.

BETHESDA UNIT OF THE LABORATORY OF BIOPHYSICS

Section on Molecular Biophysics

The Section on Molecular Biophysics is concerned with uncovering mechanisms responsible for controlling ionic current flow across axonal membranes and the influence of various chemical and pharmacological agents on these ionic movements. The Section also examines acetylcholine activated ionic channels in post-synaptic membranes. Electrical fluctuations are measured in tissue-cultured skeletal muscle cells in order to determine unit conductances of excitable ionic channels. Ionic channels in lipid bilayers are studied. In particular, alamethicin, hemocyanin and EIM (excitability-inducing material of bacterial origin) channels are examined both as to the number of conductance levels and their single channel opening and closing kinetics. A number of mathematical model systems are studied. These include models for the elastic properties of membranes, the kinetics of selective inhibition of sodium channels by drugs, and the basis for periodic bursts of impulses in bursting pacemaker neurons. One aspect of the mathematical studies is the development of interactive computer programs for solving nerve equations.

Another program of the Section involves a study of the influence of drugs and physical variables such as pressure on the voltage dependence of ionic channels in axonal and synaptic membranes. The action of drugs such as yohimbin and toxins such as Batrachotoxin are examined in detail. Another effort of the Section is to determine how and to what extent the perineurium is involved in the maintenance and regulation of the ionic and metabolic environment of the axons of peripheral nerves. Various mechanisms for water and solute permeability across the perineurium are of interest in this study.

The program of the Section has made the following advances. Studies on *Myxicola* giant axons have shown the relative permeability of sodium channels to follow the sequence: sodium > hydrazine > ammonium > guanidine > formamidine ≥ aminoguanidine >> methylamine. Studies on squid axons revealed that calcium ions temporarily block sodium channels by occupying a site halfway through the membrane field. In cultured muscle cells, the selectivity of organic molecules through acetylcholine activated channel has been shown to be charge dependent. Sodium and potassium ions have been shown to be equi-permeant in those channels. Noise studies of these channels has shown that the temperature dependent kinetics are steeper in the denervated cells than for innervated cells.

An effort was made to fuse lipid bilayers with chromaffin granules, which are storage vesicles for adrenergic transmitters using discrete conductance changes as an index. In the field of modeling, a model of the bursting pacemaker, which adds one slow recovery variable to the BVP (cubic) model of the nerve membrane, produces irregular patterns of bursting, characteristic of neurons in poor condition. A kinetic scheme of the sodium channel has been generalized to account for the action of the drug yohimbin. A study on the perineurium has resulted in an experimental method for measuring water and nonelectrolyte permeability.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01950-06 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Excitable Membrane Characteristics: Voltage Clamp and Impedance Measurements.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Adelman, Jr.	Chief	LB NINCDS
Other:	K. S. Cole	Research Biophysicist	LB NINCDS
	A. Mauro	Guest Worker	LB NINCDS
	L. DeFelice	Guest Worker	LB NINCDS
	C. Tyndale	Electronic Engineer	MBL
	R. Waltz	Mathematician Programmer	MBL

COOPERATING UNITS (if any)
Marine Biological Laboratory, Woods Hole, MA 02543

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Neural Membranes

INSTITUTE AND LOCATION
IRP, NINCDS, NIH, MBL, Woods Hole, Massachusetts 02543

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.9	2.3	2.6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general aim of this project has been to improve electrical measurements of excitable membrane characteristics consistent with physical and chemical methods for the study of nerve membrane ionic channels. Two major approaches are used. The first involves the development of methods for rapid analysis of ionic channel conductances by means of computer controlled voltage clamp techniques. Programs for carrying out this analysis are developed. The second approach involves analysis of excitable membrane characteristics by means of impedance measurements. Improvement of bridge impedance techniques to achieve greater accuracy are made. Admittance measurements on giant axons and an investigation of effects of polarizations for comparison with step and ramp techniques and ion conduction models are carried out. This project is supportive of a number of other projects in terms of the development of relevant hardware and software.

PHS-6040

Project Description:

Objectives.

(1) Rapid analysis of ionic channel conductances by means of computer controlled voltage clamp: to develop modern approaches and systems for controlling voltage clamp experiments and acquiring excitable membrane conductance data in a form that can be analyzed by digital computing machines; to develop programs for carrying out this analysis.

(2) Steady-state analysis by means of impedance measurements: the improvement of bridge impedance techniques and accuracy on axon membranes and investigation of effects of polarizations for comparison with step and ramp techniques and ion conduction models.

(3) Steady-state analysis by means of small signal admittance measurements.

Methods Employed:

(1) The general method has involved the integration of general purpose data acquisition equipment into dedicated systems, suitable for controlling and acquiring data from biophysical and neurophysiological experiments.

(2) An impedance bridge with internal guarded electrodes has been used and a new impedance bridge employing guarded external electrodes has been developed.

(3) Small signal voltage clamp admittance measurements are made on squid giant axons.

Major Findings:

(1) The computer controlled data acquisition system has been redesigned and constructed at the Marine Biological Laboratory in Woods Hole. This systems makes use of a PDP-11/10 digital computer, with four floppy disks and two 9-track read-write magnetic tape units as peripherals. An electrostatic line printer has been added. The system is also interfaced via telephone line to large number crunching computers at DCRT in Bethesda and SIGMA 7 at Woods Hole Oceanographic Institution (WHOI) in Woods Hole. An operating program for voltage clamping axons has been designed and constructed. This system makes use of high speed 10 bit A/D and D/A converters. The software for program control has been developed and the system is now operational.

(2) The underground bridge assembled for guarded impedance measurements of axons has been delayed. The gyrator circuits for the inductive reactance were ordered two and one half years ago as integrated units, but were not delivered. The assembled circuit components were unstable, but the recent appearance of the integrated circuits makes it possible to continue with the axon problem. It is hoped to determine whether or not the apparent membrane capacity loss is real or an artefact of less than ideal measurement geometry.

(3) At 8°C an antiresonance in the squid axon membrane admittance-frequency relation has been found with a center frequency at about [illegible] Hz when the membrane is slightly depolarized in the steady state. At the antiresonance frequency, the membrane current oscillates at twice the frequency (1[illegible]) of the small signal sive wave impressed on the steady depolarizing voltage. The results are in qualitative agreement with predictions obtained from solutions of the Hodgkin-Huxley nerve equations.

Publication:

Cole, K.S.: Membranes. Ann. Rev. Physiol., 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02087-04 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Adelman, Jr.	Chief	LB NINCDS
Other:	R. J. French	Visiting Associate	LB NINCDS
	J. B. Wells	Research Physiologist	LB NINCDS
	J. J. Shoukimas	IPA Fellow	LB NINCDS

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Neural Membranes

INSTITUTE AND LOCATION
IRP, NINCDS, NIH, MBL, Woods Hole, Massachusetts 02543

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	3.3	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Voltage clamp experiments are carried out to determine the function and structure of ionic channels in single giant nerve fibers. Information is obtained about the position and properties of membrane ionic channel sites that limit conductances and determine ionic selectivity by an analysis of the interaction between current-carrying and blocking ions. The ability of various kinetic models to describe the flow of ions through open potassium channels is tested. Kinetics of ionic blocking of channels are studied to gain information about energy barriers crossed by ions entering channels, and about the nature of ion binding sites within the channels. Analogies between the kinetics of ionic currents across the nerve membrane and those seen in excitable lipid bilayer membrane preparations treated with channel forming agents are investigated. The effect of calcium ions upon sodium channel inactivation is studied.

Project Description:

Objectives.

(1) To describe the interactions between various univalent ions and the K channel of squid axon and provide a descriptive basis for quantitative rate theory modeling of the open channel.

(2) To study the effects of external calcium upon sodium inactivation in the squid axon and compare results with various models of calcium action.

Methods Employed.

(1) Ion interactions in K channels. Both intact and perfused axons have been studied under voltage clamp. Effects of internal ions have been studied in two types of experiments: a. using a single cation species within the axon. Sodium, potassium, rubidium, cesium and ammonium have been used as test ions. b. other cations have been added in the presence of the normal (300 mM) concentration of K. Sodium, cesium, rubidium, ammonium, methylammonium and tris (hydroxymethyl) aminomethane have been examined in this way.

(2) Effects of Ca upon Na inactivation. The effect of varying concentration of calcium upon sodium inactivation was studied by means of a prepulse - test pulse voltage clamp regime.

Major Findings.

(1) Ion interactions in K channels. Methylammonium and tris (hydroxymethyl) aminomethane both produce a voltage dependent block of K channel currents somewhat like that caused by internal sodium and cesium.

With internal sodium on cesium in the presence of 300 mM K, the current voltage (I-V) relation is N-shaped. It turns upward at about V = 120 to 160 mV (inside minus outside). With only sodium or cesium within the axon, the I-V curve bends upward at about the same voltage, while at lower voltages there is little or no measurable current. Additional experiments with internal sodium suggest that the K channel has a significant permeability to Na ions above $V \simeq 150$ mV.

A Manuscript describing the blocking of the K channel by external Cs has been submitted for publication.

(2) Ca effect on Na inactivation. The effect of calcium was found to be consistent with a charge screening theory for steady state inactivation behavior, but kinetic effects were in strong disagreement with thie theory. Calcium and magnesium do not behave as equimolar substitutes which is a new finding in disagreement with work on other preparations.

Proposed Course of Project:

1. Ion interaction in K channels.

Block of the K channel by alkali cations usually appears to be effective instantaneously after a voltage clamp step. The I-V studies reveal [illegible] conditions under which it may be possible to observe directly the [illegible] kinetics following a voltage step. These will be investigated. Preliminary studies on the effects of temperature on the blocking reactions [illegible] be extended.

2. Ca effect upon Na inactivation.

A more complete study of the effects of Mg^{++} ions is planned for comparison with present data. In addition, kinetic studies on *Myxicola* axons suggest that more than one process may be required to fully characterize sodium inactivation over short durations. This possibility and the effect of divalent cations will be explored in the squid axon.

Publications:

1). French, R.J. and Wells, J.B.: Sodium ions as blocking agents and charge carriers in the potassium channel of the squid giant axon. J. Gen. Physiol. (in press).

2). Shoukimas, J.J.: The effect of calcium upon sodium inactivation in the giant axon of *Loligo pealei*. J. Membrane Biol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02092-04 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Subcellular and Extracellular Structure Associated with Nerve and Muscle.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Adelman, Jr.	Chief	LB NINCDS
Other:	K. S. Cole	Research Biophysicist	LB NINCDS
	R. V. Rice	Guest Worker	LB NINCDS
	P. F. Roslansky	Guest Worker	LB NINCDS
	J. Shoukimas	IPA Fellow	LB NINCDS
	V. Sege	COSTEP Officer	LB NINCDS
	J. Moses	Research Assistant	Carnegie-Mellon U.
	A. Cornell-Bell	Research Assistant	Carnegie-Mellon U.

COOPERATING UNITS (if any)

Carnegie-Mellon University, Pittsburg, Pa. 15213
Marine Biological Laboratory, Woods Hole, MA 02543

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Neural Membranes

INSTITUTE AND LOCATION
IRP, NINCDS, NIH, MBL, Woods Hole, Massachusetts 02543

TOTAL MANYEARS: 4.2	PROFESSIONAL: 3.2	OTHER: 1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to examine the subcellular and extracellular structure of nerve and muscle and relate such structure to function. Electron microscopy, protein chemistry, structural modeling, physical chemistry and electrophysiological methods are employed. The following structures are probed:

1) Cytoskeleton, 2) Neurofilaments, 3) microtubles, 4) Schwann sheath,

5) Schwann cells, 6) Endoneurium.

The Schwann sheath and generalized neuronal packing are modeled. In addition, the ionic composition of squid hemolymph is examined.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives.

One of the objectives of this project was to examine certain proteins in the cytoplasm of nerve and muscle cells. Neurofilaments have long been recognized as structural components of nerve cells. In the giant axon of the squid *Loligo pealei*, they lie parallel to the long axis of the axon and measure 10 nm by several μm. They represent an intermediate filament, being larger than action filament which measure 6 to 8 nm in diameter, and smaller than microtubules which have an average diameter of 24 nm. Intermediate filaments form a cytoskeletal network in many cells.

These filaments have been examined using ultramicroscopic and biochemical techniques. Neurofilaments, 10 nm in diameter, obtained from extruded axoplasm of the squid, *Loligo pealei*, were concentrated by both low speed centrifugation and a combination of sonication and milliport filtration. Using gel electrophoresis, the major proteins in the concentrated suspensions were shown to include a 200,000 dalton protein which comigrated with rabbit myosin, and proteins of molecular weights of 160,000 and 63,000. In filtration experiments, the 63,000 dalton protein was not only concentrated in the retentate, but also removed from the filtrate. Using the Fairbanks gel system, this protein migrated slower than brain tubulin. A paper describing these results has been submitted for publication. A second objective of this project was to correlate periaxonal tissue resistance with Schwann cell layer anatomy. Cross and longitudinal sections of giant axons of *Loligo pealei* were examined by transmission electron microscopy. Measurements were made of the width and frequency of mesaxonal clefts entering the Schwann cell layer from the periaxonal space and exiting the cell layer into the basal lamina. The average mesaxonal cleft width = 10.5 nm and the Schwann cell layer was shown to contain about 120,000 Schwann cells per cm^2 of axon surface. This cell layer was shown to have an extracellular volume of 6.5×10^{-7} cm^3 or 0.7% of the Schwann cell layer volume. Several volume conductor models were derived so as to predict the value of the resistance across the Schwann cell layer. The value of the Schwann cell membrane capacity was also estimated. Both resistance and capacitance predictions were in the range of physiological estimates. A paper describing these results has been accepted for publication.

Another aspect of this project was the analysis of tissue impedance. Analogue solutions for low frequency resistance of several close packing two and three dimensional forms and for low frequency capacity of square cylinders in square array have been reported for volume concentrations from 0 - 100% and agreed with LaPlace equation analytical solutions for low concentrations. The similar problem for low frequency capacity of close-packing three dimensional forms could be anticipated and it was expected that its solution would similarly extend the range of the LaPlace solution.

Bridge measurements on one quarter of a membrane covered cube in electrolyte between electrodes in a V trough confirm this expectation. The data usually did not require the available correction for electrode polarization and the results include an empirical form factor which should be tested by analysis.

Another objective of this project was to determine the ionic composition of squid hemolymph. Hemolymph and protein-free hemolymph obtained from *Loligo pealei* were analyzed for cation concentration by the method of atomic absorption spectroscopy for the following ions: Na^+, K^+, Ca^{++} and mg^{++}. No significant differences were seen in ion concentration between hemolymph and protein-free hemolymph.

The average values were: Na=393 mM, K=11.7 mM, Mg=45.9 mM and Ca=10.4 mM. Of particular neurophysiological significance is that K concentration is much closer to that of seawater than previously reported.

Publications:

Adelman, W.J., Moses, J. and Rice, R.V.: An anatomical basis for the resistance and capacitance in series with the excitable membrane of the squid giant axon. J. Neurocytology (in press), 1977.

Cole, K.S.: Resistance and capacity of a cubic array of membrane-covered cubes at high concentration. Proc. Nat'l Acad. Sci., USA. 73: 4003-4006, 1976.

Shoukimas, J., Adelman, W. J. Jr. and Sege, V.: cation concentrations in the hemolymph of *Loligo pealei*. Biophysical J. 18: 231-234, 1977.

ITHSONIAN SCIENCE INFORMATION EXCHANGE
ROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02252-01 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Visual pigments: a comparative study of chemical properties.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. I. Harosi	Research Associate	LB, NINCDS
Other:	E. F. MacNichol	Director	LSP, MBL
	M. A. Ali	Professor, Biology	U. Montreal
	H. -J. Wagner	Assistant Professor, Anatomy	U. Ulm
	C. Sandorfy	Professor, Chemistry	U. Montreal
	H. Shichi	Chemist	LVR, NEI

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Neural Membranes

INSTITUTE AND LOCATION
IRP, NINCDS, NIH, MBL, Woods Hole, Massachusetts 02543

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is aimed at attaining a detailed understanding of the structural and functional relationships of photoreceptors and their visual pigment content pertaining to photic excitation. Topics of current study are:

1) Spectroscopic characterization of vertebrate visual pigments in situ.
2) Description of photoreceptors for visual pigment content, receptor mosaic, habitat, and evolutionary trends in bony fishes.
3) Theoretical and experimental study of the band structure of the absorption spectra in visual pigments and related molecules.
4) Bleaching kinetics studies in solubilized visual pigments from the viewpoint of solvent effects.
5) Chemical probing of the micro-environment of the visual pigment chromophore in cells and in solubilized pigments with dissolved dyes and organic molecules.

Project Description:

Objectives. The ultimate purpose of this project is to attain a detailed understanding of the physical (structural) and chemical (functional) relations in visual pigments pertaining to photic excitation. Topics of current study are:

1) Spectroscopic characterization of vertebrate visual pigments in situ.

2) Description of photoreceptors for visual pigment content, receptor mosaic, ecologic factors (habitat), and evolutionary trends in bony fishes.

3) Theoretical and experimental study of the band structure of the absorption spectra in visual pigments and related molecules.

4) Investigation of the role of phospholipids in extracted, solubilized, visual pigments; their effect upon regeneration and bleaching kinetics.

5) Chemical probing of visual pigments in cells and in solutions by the use of lipophilic agents, fluorescent dyes, inorganic ions (vir., chloride ions vs gecko rhodopsin), and organic molecules.

Methods employed.

1) Absorption spectroscopy of visible and ultraviolet light on,

   a. single cells, and
   b. solutions..

2) Light and electronmicroscopy,

3) Chemical techniques (chromatography)

4) theoretical modeling (computer analysis).

Major Findings:

1) In the cichlid fish, *Nannacara anomala*, we found four visual pigments: one in the rods and three in the cones. The trichromatic cone system in this retina was not to be expected on the basis of organization, for there are only two cone-horizontal cell layers in it. The cones are arranged in a regular square mosaic consisting of four pairs of double cones along the sides and a small single cone in the middle. The single cones were found to contain only blue-absorbing pigment (peaking at about 455 nm). The double cones were found to have two spectrally distinct pigments: one member absorbing in the yellow (555 nm), the other in the more reddish (600 nm) part of the spectrum. The rods, which are relatively thick in this fish and hence yield more accurate spectral data, were found to contain a pigment with a spectral peak of 498 nm. Based on spectral band-width determinations, the four visual pigments were identified as predominantly porphyropsin type, with the possible admixture of some (perhaps 10%) rhodopsins (collaboration with Ali and Wagner).

2) Three species of fish from the *Perca* family were studied: yellow perch, walleye and sanger. Retinas from these fishes contain numerous but [illegible] thin rods, single cones, and mostly equal double cones with large ellipsoids. Preliminary results show that both members of double cones contain spectrally indistinguishable pigments absorbing in the yellow-orange spectral range. (collab. with Ali).

3) Cattle rhodopsin solubilized in aqueous digitonin and purified by column chromatography resulted in three fractions. The first fraction-peak was obtained by elution with digitonin-containing buffer. The second and third fraction-peaks were eluted with a NaCl gradient and digitonin buffer. The rhodopsin of fraction-peak one retains high regenerability and its photoproducts (metarhodopsin III) decay slowly whereas the second and third fractions have low regenerability and fast decay. Since phospholipids have been found to play a stabilizing role in rhodopsin, the purification technique thus developed appears to be useful for such study. The chemical characterization (lipid composition) of the three fraction-peaks remains to be done. (Collab. with Shichi and his co-workers).

Significance to Biomedical Research:

The knowledge of visual pigment absorption spectra is fundamental for physiological and behavioral studies. Evolution and ecologic correlations may be important in understanding how and why the great variability in visual pigments evolved. Learning the way the spectral properties are varied in nature should be helpful in understanding the deficiencies (anomalies) in human color vision. Visual excitation (properties of visual pigments) and retinal processing of information (inter-connections of retinal neurons) are fundamental aspects of biology.

Proposed Course of the Project:

1) Continue spectroscopic studies of visual pigments; extend these to new vertebrate species (lamprey, skate) and to invertebrates (squid, *Hermissenda*).

2) Continue the comparative study of visual pigments from viewpoints of ecology and evolution.

3) Pursue theoretical spectroscopy to elucidate band structure.

4) Pursue chemical probing of visual pigments to elucidate mode of action of absorption-excitation coupling.

5) Continue the study of the role of phospholipids in rhodopsin.

Publications:

Harosi, F.I.: Spectral relations fo cone pigments in goldfish. J. Gen. Physiol. 68: 65-80, 1976.

Stell, W.K. and Harosi, F.I.: Cone structure and visual pigment content in the retina of the goldfish. Vision Res. 16: 647-657, 1976.

Shichi, H., Harosi, F.I., Kawamura, S. and Yoshizawa, T.: Isolation of three isochromic forms of rhodopsin which show different bleaching kinetics. Biophys. J. 17: 79a, 1977.

Shichi, H., Muellenberg, C.G., Harosi, F.I. and Somers, R.L.: Isolation of three isochromic forms of rhodopsin in digitonin. Vision Res. 17:633-636, 1977.

Harosi, F.I.: Comments on form dichroism in vertebrate photoreceptors. Vision Res. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	Z01 NS 02272-01 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Transmembrane potentials: Effect of unstirred layers near ion exchange membranes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. French	Research Associate	LB, NINCDS

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Neural Membranes

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of the project was to identify the reason for reported discrepancies between the experimentally measured transmembrane voltage across an ion exchange membrane and the predictions of the Nernst-Planck equations for ionic diffusion. Numerical methods of solution were used to eliminate the possibility that significant errors were introduced by the application of the constant field assumption. The computed Donnan potentials across the membrane-solution interfaces were extremely sensitive to the assumed thickness of the unstirred solution layer adjacent to the membrane. When this was taken into account, it was possible to fit the experimental data. Similar effects could occur in experiments with freshwater algae which have been widely used for electrophysiological studies.

Project Description:

Objectives.

To describe the potential difference across an ion exchange membrane using the Nernst-Planck equations for ionic diffusion. To investigate the effects of unstirred layers in the solution adjacent to the membrane on the predicted diffusion potential.

Methods Employed:

The experimentally measurable potential difference was considered to have five components: a diffusion potential in the unstirred solution layer on each side of the membrane. Data from the literature, reported as incompatible with an electrodiffusion equation description, were fit and model calculations were carried out.

Major Findings.

The Donnan component of the potential is extremely sensitive to the unstirred layer thickness when a highly charged ion exchange membrane is in contact with a dilute solution. The Donnan components represent the major fraction of the total transmembrane potential. Consequently, the unstirred layers have a significant influence on the measured potential difference. Similar effects are predicted for some freshwater biological preparations.

Proposed Course of Project: Project terminated.

Publication:

French, R.J.: Unstirred layer effects on calculations of the potential difference across an ion exchange membrane. *Biophys. J.* 18: 53-61, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02273-01 LB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

An Investigation of Electro-mechanical Coupled Interaction in Excitable Tissues.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Wells	Research Physiologist	LB, NINCDS
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work examines the effect of mechanical perturbations on the ionic conductance processes of the squid giant axon. Controlled reversible disturbances are applied to the surface membrane and subsequent changes in the electrical parameters are measured and analyzed.

Objectives.

The main objective is to measure and define certain mechanical characteristics of excitable tissues and to determine their interactions with ionic conductance phenomena. Special attention will be paid to longitudinal and radial components of compliance and its viscoelectric nature. Studies will be carried out of electrical responses to the application of mechanical forces.

Methods Employed.

Instrumentation has been assembled which includes a sensitive straingauge transducer for compliance measurements and a servo-regulated motor for the application of controlled mechanical perturbations to the tissues studied. Also included is apparatus for recording appropriate electrical and mechanical events.

Major Findings.

a) Past work - Anticipated results from this study are based on old observations that sufficient mechanical disturbance of the surface of excitable cells will result in a depolarization of the membrane which may generate an action potential.

b) Present work - Since preliminary experiments on this project have only recently begun, there are no major findings to report.

Proposed Course of Project:

The establishment of a reversible standardized mechanical disturbance of the surface membrane and its contained ionic conductance mechanisms is required to permit detailed analyses of the underlying processes involved. Such environmental factors as temperature, ionic composition of bathing solutions and the presence of certain drugs may then be used to characterize the mechanisms involved in the electro-mechanical interactions.

Publications:

None.

NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 NS 02151-03 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Information Processing in Simple Nervous Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	T. Crow	Extramural Fellow	LB NINCDS
	Y. Grossman	Visiting Fellow	LB NINCDS
	I. Lederhendler	Visiting Fellow	LB NINCDS
	J. Harrigan	Research Associate	MBL
	L. J. DeFelice	Associate Professor	Emory U.
	W. Rall	Guest Worker	LB NINCDS
	J. Rinzel	Guest Worker	LB NINCDS
	R. Miller	Guest Worker	LB NINCDS
	S. Lehky	Guest Worker	U. of Chicago

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	7.5	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal objective of this project is to study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. The nervous system of *Hermissenda crassicornis* has proven to be an excellent model for information processing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in synaptic circuitry produced by conditioning paradigms administered to intact animals as well as to isolated nervous systems, membrane properties modified by conditioning, identification of critical developmental stages for the neural networks studied as well as stages critical for learning. Techniques employed thus far to pursue these questions include: simultaneous intracellular recording from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophonesis of fluorescent dyes and electron dense materials, automated behavioral monitoring of intact *Hermissenda*. Other methods include mariculture, subcellular fractionation protein phosphorylation analysis, and uptake of neurotransmitter precursors.

Project Description:

Objectives:

(1) To study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. Information processing at several levels is of interest:

a. Sensory transduction by photoreceptors and hair cells.
b. Synaptic interactions between primary sensory receptors.
c. Synaptic interactions between primary and higher order neural elements.
d. Intersensory communication: e.g. synaptic interaction between the visual and gravitational sensory pathways.
e. Changes of synaptic interaction produced by conditioning paradigms administered to the intact animals as well as to the isolated nervous systems.
f. Membrane and synaptic properties modified by conditioning.
g. Identification of critical developmental stages of the neural networks studied as well as stages critical for learning.

Methods Employed.

(1) The nudibranch mollusc *Hermissenda crassicornis* is the principal experimental preparation. Other marine species are also being screened to provide favorable preparations for specific experimental questions. Intracellular recording from several neural cells simultaneously has been the main technique used thus far. Means for simultaneously stimulating the visual and vestibular pathways (which has permitted conditioning) while recording intracellular potentials have been developed in our laboratory. Iontophonesis of fluorescent dyes (e.g. Procion Yellow) and electron dense materials (e.g. cobalt) are also being used extensively.

(2) Other methods allow biochemical and developmental approaches to the problems of interest. These include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, etc. Automated behavioral monitoring now permits long-term studies of intact *Hermissenda*.

Major Findings:

(1) Past work has focused on four major areas:

a. Receptor physiology.
b. Neural network analysis.
c. Behavioral conditioning with neural correlates, and
d. Cellular conditioning in isolated nervous systems.

(2) Hair Cell Physiology: With an improved rotatory stimulus it has been possible to characterize more exactly the nature of adequate stimulation of hair cells. Specifically, hair responses to changes in angular

acceleration could be carefully compared to responses to changes in angular velocity. Stimulus threshold, the role of adaptation, [illegible] relationships, and hair cell noise have also been carefully [illegible].

Within the past year visualization of moving hairs has been accomplished with sufficient resolution to allow quantification of movement frequency with [illegible] stroboscope. Strict correlation of hair movement frequency with hair cell voltage noise and generator potentials has provided the data for a conceptual model of mechanotransduction. This model holds that both voltage noise and generator potentials arise from collision of statoconia with hairs. The establishment of this common origin has led to estimation by noise analysis of elementary conductance events unique to the mechanotransduction process.

(3) Cellular Conditioning: Stimulus paradigms have been developed for stimulation of the visual and vestibular pathways in isolated *Hermissenda* nervous systems. These paradigms can now be characterized as conditioning paradigms because of their required temporal specificity, their cellular specificity, and the long-lasting neural changes they produce. Type A but not Type B photoreceptors respond with fewer impulses to a light stimulus following repeated temporally specific pairing of light and rotation. This conditioning effect can now be explained by a mechanism involving a long-term network bias: Type B photoreceptor inhibition of Type A cells is enhanced by pairing the inhibitory effect (through the hair cells) of rotation with the exciting effect of light. This network bias was further demonstrated by simultaneously recording from the pre- and post-synaptic elements, the Type B and A photoreceptors.

An understanding of the basis for the cellular and behavioral conditioning previously identified for intact animals as well as their isolated nervous systems has been extended within the past year by observation of long-lasting excitation and inhibition in the eye of *Hermissenda*. The excitation, associated with a long-lasting increase of membrane resistance, appears to be unique to the phototransduction process in these cells. The inhibition, in a single photoreceptor in each eye was shown to be a synaptic phenomenon and to arise as a direct consequence of long-lasting excitation of Type B cells and an indirect consequence of increased firing of specific ipsilateral hair cells.

(4) Non-synaptic neuronal interaction: We have recently established that excitation of hair cells by Type A photoreceptor impulses persists during synaptic blockade. No resistance change was observed during this excitation when synaptic blockade is effected by perfusing with Co^{++} or Co^{++} with reduced extracellular calcium. Additional data were also obtained which support the interpretation that the excitation of hair cells by Type A impulses arises from potassium accumulation around the Type A photoreceptor and hair cell axonal membranes. As such, this represents the first well-documented case of neurons exchanging significant information without either electrical or chemical synapses.

(5) Electronmicroscopic Identification of Synaptic Junctions: Techniques for iontophoretic injection of the electron dense substances, cobalt and horseradish peroxidaxe, have been successfully adapted for identifying

synaptic junctions in *Hermissenda*. Histologic processing, involving both histochemical and cryostatic methods, has also been developed. Within the past year, neurons within the *Hermissenda* nervous system have been shown to have adjoining terminal axonal membranes in close apposition when these neurons have by electrophysiological criteria been shown to make monosynaptic connections. This demonstration of synaptic interaction with the electron-microscope utilized iontophoretic injection of horseradish peroxidase into pre- and post-synaptic neurons.

This use of electronmicroscopic combined with electrophysiologic data now provides for the first time rigorous criteria for the identification of synapses in molluscan nervous systems.

(6) Biochemical Analyses: Neurotransmitter substances have been identified and characterized in the isolated circumesophageal nervous system as well as isolated structures such as the eyes, statocyst, and optic ganglia of *Hermissenda*. Uptake of radioactive precursors and synthesis of neurotransmitter substance, particularly acetylcholine, have been observed in these structures.

When isolated eyes were incubated with labelled precursors, only acetylcholine was synthesized and accumulated at detectable levels (1-5 pmoles). Intense histochemical staining for acetylocholine-sterase was observed in the region of the photoreceptor terminal branches. Histofluorescence localization of DA and 5-HT indicated that only one second order visual cell within each optic ganglion contained dopamine. Clear round vesicles were observed in endings of identified photoreceptors injected intracellularly with horseradish peroxidase. Intracellular recordings from identified photoreceptors were made while the nervous system was perfused with neurotransmitter agents: carbachol, dopamine, 5-hydroxytryptamine, GABA, histamine, and glycine. Carbachol produced hyperpolarization of 5-15 mV accompanied by a drop of 10-60% in input resistance. The reversal potentials for carbachol as calculated from the IV curve (-70 to -75 mV for Type A, and -85 to -90 for Type B) were similar to that of IPSP's which followed, one for one, neighboring photoreceptor impulses elicited either by light or intracellular current injection. Single cell analyses are now being prepared. These will be performed in conjunction with other experiments involving iontophoretic injection of radioactive precursors and metabolites in single cells.

(7) Behavioral Experiments: An automated apparatus for long-term behavioral monitoring has been assembled. This will permit testing of conditioning paradigms on intact *Hermissenda* over many weeks and possibly months. The proper natural environment for continued maintenance of animals to be tested in these experiments can now be provided utilizing the seaside holding facilities of the Marine Biological Laboratory.

With the automated apparatus it has recently been possible to demonstrate features of learning in the relatively unevolved gastropod *Hermissenda* which resemble learning in the much more highly evolved vertebrate species. Thus, long-term (many days) training of *Hermissenda* was shown to specifically result from paired visual and vestibular stimuli, just as had previously been demonstrated for short-term (several hours training). In addition, characteristics observed for the long-term training now include: acquisition, retention, savings and extinction. These common features of vertebrate learning and learning in *Hermissenda* suggest that the cellular mechanisms we are defining in a relatively simple neural network have some generalizability and perhaps ultimately may have clinical significance.

(8) Mariculture: *Hermissenda* larvae have been successfully raised to the final premetamorphosis stage. Triggering substances are now being screened for matamorphosis induction. Nutrients have also been screened for adult animals and *Hermissenda* shipped from the Pacific grow considerably with the feeding and maintenance procedures developed in the last several months.

Within the past year a substantial number of *Hermissenda* (as well as other related nudibranchs) have been successfully reared from fertilized eggs to post-metamorphosis juveniles. Thus, genetically defined laboratory strains of *Hermissenda* are now becoming a reality.

(9) Other related findings:

a. Electrophysiologic analysis of the eye of *Elysia*, a nudibranch mollusc indigenous to Cape Cod, has been performed. These photoreceptors, (approximately 15 in number) in response to light, depolarize with an associated decrease in membrane resistance. Their synaptic interactions, in many respects similar to those of *Hermissenda*, are inhibitory and occur approximately 2 cm from the somata. The photoreceptor impulses also arise within the distal axon and only passively invade the somata.

b. The interaction of chemosensation with sensory perception mediated by the visual and statocyst pathways were investigated. Responses to natural stimulation of the tentacles, isolated intact with the circumesophageal nervous system, by food substances and to electrical stimulation of the tentacular nerves were recorded intracellularly from neurons in the cerebropleural ganglia, photoreceptors in the eye, optic ganglion cells and statocyst hair cells. Neurons in each of these structures were found to respond in specific ways to stimulation of the chemosensory pathway originating at the tentacular chemoreceptors as well as to stimulation of the visual pathway originating at the photoreceptors. Synaptic inhibition of photoreceptors was demonstrated to occur via a chemosensory pathway. The functional significance of this new example of intersensory interaction in *Hermissenda* (visual-vestibular interaction has been defined in previous work) was investigated with reference to the animal's gustatory behavior and possible neural mechanisms of behavioral choice.

Proposed Course of Project:

(1) Precise analysis of synaptic interactions between cells within the aforementioned neural networks will be continued using the techniques of intracellular recording and iontophoresis. Particular emphasis will be placed on electronmicroscopic visualization of cell contacts aided by axonal

absorption of hydrogen peroxidase and/or cobalt. These studies will not be limited however, to the networks already discussed. A third sensory pathway that mediating chemoreception, will also be mapped. In addition, other marine forms with potentially analyzable neural networks and behavior will be explored.

(2) Detailed noise analyses will be conducted on hair cell potentials with and without physiologic stimulation. Efforts will be made to identify the behavior of membrane ionic channels responsible for stimulus transduction in the hair cells.

(3) Cellular mechanisms responsible for the learning identified in the intact animal and its isolated nervous system will be further analyzed. Careful attention will be given to synaptic processes responsible for the long-term inhibition believed to underly cellular conditioning.

(4) Biochemical and pharmacologic analyses of neurotransmitter substances within the visual and vestibular pathways will be continued. Studies are also planned to identify subcellular and/or biochemical loci for the neural changes produced by the conditioning paradigms used.

(5) Behavioral experiments will be continued to ascertain the comparability of cellular conditioning and behavioral conditioning as well as the comparability of changes observed for *Hermissenda* to changes observed for animals with more evolved nervous systems.

(6) *Hermissenda* will be raised through successive generations. It is planned to combine all of the above approaches with mariculture. The existence of clearly identified neural networks in *Hermissenda* together with mariculture offer for the first time the possibility of using genetic mapping to study principles of neural organization, development, and learning.

(7) The many marine forms available at the Marine Biological Laboratory (MBL) will be screened to identify other neural networks and synaptic junctions. The generalizability of principles and phenomena observed in *Hermissenda* may then be tested and compared for other evolutionary stages.

Publications:

Alkon, D.L.: Neural Modification by Paired Sensory Stimuli. The Journal of General Physiology. 68: 341-358, 1976.

Alkon, D.L.: The Economy of Photoreceptor Function in a Primitive Nervous System. F. Zettler and R. Weiler (Eds.): Neural Principles in Vision. Berlin Heidelberg, Springer-Verlag, 1976, pp. 410-426.

Harrigan, J.F. and Alkon, D.L.: Laboratory Cultivation of *Haminoea Solitaria* Say, 1822 and *Elysia Chlorotica* Gould, 1870, The Veliger, 1977 (in press).

DeFelice, L.J. and Alkon, D.L.: Voltage Noise from Hair Cells during Mechanical Stimulation. Nature, 1977 (in press).

Alkon, D.L., Grossman, Y. and Akaike, T.: Non-synaptic Neuronal Interaction. J. Neurophysiology, 1977. (in press).

Crow, T.J., Finch, D.M. and Babb, T.L.: Absence of Cerebellar Influences on Hippocampal Neurons in the Cat.[1]. Exp. Neurol., 1977 (in press).

Lederhendler, I.I.: The development of conspecific interactions in juvenile *Aplysia dactylomela*. The Veliger, 1977. (in press).

Lederhendler, I.I., Herriges, K., and Tobach, E.: Response to conspecific *Aplysia dactylomela* by taxis. Animal Learning and Behavior, 1977. (in press).

Petrinovich, L. and Crow, T.J.: Spreading Depression and Memory Transfer. Behavior Biol., 1977 (in press).

Alkon, D.L., Akaike, T. and Harrigan, J.S.: Interaction of Chemosensory, Visual and Statocyst Pathways in *Hermissenda Crassicornis*. J. Gen. Physiol, 1977. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02088-04 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

E OF PROJECT (80 characters or less)

Function and Structure of Membrane Ionic Channels: Pharmacology and Ionic Selectivity*

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS: 3.6	PROFESSIONAL: 2.0	OTHER: 1.6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range purpose of this project is to study the function and structure of membrane ionic channels. The areas of present research are:

1) The permeability of organic cations in the sodium channel of Myxicola;
2) The influence of ionic strength on ionic selectivity.
3) The effects of neurotoxins on the axonal membrane in Myxicola;
4) The kinetics of various ions (mainly calcium, magnesium and cesium) through the sodium channels of the squid giant axon;
5) The influence of charge on ionic permeability in tissue-cultured cells derived from chick embryo muscles.

Project Description

Objectives: To study the ionic current flow across the nerve membrane without the complication of excitation and propagation in terms of individual ion currents and to observe the effect of changes in normal external and internal environments. These environments are altered by addition of various chemical and pharmacological agents as well as by changing the ionic environment. Also to study the effect of charge of the transporting molecules on the rate of their permeation through acetylcholine channels. The long range objectives are the interpretations of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed on preparations a and b: (a) *Myxicola* giant axon. For many experiments on the giant axon of *Myxicola*, the voltage clamp is under computer control; (b) Squid giant axon. The squid giant axon is internally perfused and signal averaging techniques are used. Standard radioactive counting techniques are employed with preparation (c): (c) Cultured muscle cells from chick embryo. Carbamylcholine is used to open the acetylcholine channels in these cells, and radioactive tracers are used to measure ionic uptake.

Major Findings: (a) *Myxicola* giant axon: The relative permeability of sodium channels to organic cations, both permeant and impermeant, was determined. The permeability sequence was found to be: sodium > hydrazine > ammonium > guanidine > formamidine ≥ aminoguanidine >> methylamine. Methylated cations were found to be impermeant. The relative permeabilities of the cations were independent of concentration. The structure of the sodium channel seems to be quite similar to the sodium channels of other preparations, although they are distantly related.

Preliminary dose response data for the neurotoxin, veratridine, has been obtained. The apparent decrease in potassium has been shown to be caused by a steady state sodium current. Tetrodotoxin restores the decrease of potassium produced by veratridine. Batrachotoxin and aconitine have been more difficult to study. The best results for the study of the neurotoxins were obtained in potassium-free seawater with low calcium and low magnesium.

(b) Squid giant axon: External calcium ions interfere with the movement of sodium ions through the sodium channel. Much more data were fitted by a model which assumes that a calcium ion temporarily occludes the channel when it occupies a site about halfway through the membrane field. A manuscript, for publication, is in preparation.

(c) Cultured muscle cell: The permeability of 15 organic molecules through the acetylcholine channels was measured. It was shown that the selectivity of these molecules is mainly determined by the charges on the molecule. Hydrogen-bonding ability of the molecules also affect their permeation. The uncharged molecules to a lesser degree go through the acetylcholine channels.

Potassium ion is just as permeable as the [illegible] ore in the postsynaptic membrane. Change in pH of the bathing solution has a very similar effect on both sodium and potassium. A manuscript for publication is in preparation.

Significance to Biomedical Research and the Program of the Institute: The determination of the relative permeabilities of different ions through sodium channels of Myxicola provides information to test hypotheses that the sodium channel is lined with oxygen atoms. Furthermore, the results strongly suggest that widely differing species have sodium channels that are very similar. This suggests a universal selectivity filter, possibly part of a membrane protein.

The result that calcium ions can bind at a particular site in the sodium channel not only provides information about the channel, but also offers a possible method for blocking sodium channels.

The result that charge plays a crucial role in determining ionic permeability is also of considerable value in determining the mechanism for cationic selectivity in channels. In particular, this result shows that the cation selectivity does not depend primarily on cation size (cations tend to be significantly smaller than anions), but on the electrical charge itself.

Proposed Course of Project: Study mixtures of organic cations and sodium for competition. Continue the study of the following toxins: veratridine, batrachotoxin, aconitine, and scorpion venom. Myxicola axons will be voltage clamped to study the interactions of these toxins in order to: (1) Observe the ionic currents; (2) Study the kinetics to see if there are any changes in the membrane; (3) Determine if the neurotoxin sites are related to the gating mechanism, by measuring gating currents before and after application of the toxins. For acetylcholine channels in cultured muscle cells it is planned (1) to continue the study of the effect of pH on the sodium channels, (2) to study the permeability of several uncharged molecules through sodium channels in neuroblastoma N18 and C9 clones. This is to find if the permeability characteristics are different in the cells which have different responses to tetrodotoxin.

Publications:

Binstock, L.: Permeability of the Sodium Channel in *Myxicola* to Organic Cations. *J. Gen. Physiol.* 68: 551-562, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02089-04 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Fluctuation Phenomena in Excitable Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Lecar	Research Physicist	LB NINCDS
Other:	G. Weiss	Mathematician	DCRT
	R. McBurney	Visiting Associate	LN NINCDS

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TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Agonist-induced electrical noise is measured in excitable cells grown in tissue culture. The initial studies of acetylcholine and carbachol induced noise in skeletal muscle cells characterized the postsynaptic ionic channels. A collaborative experiment is in progress to measure glutamate and GABA induced fluctuations in tissue cultured CNS neurons. Theoretical studies of stochastic models of the gating process are directed towards understanding the conformation changes in membrane proteins underlying nerve excitation (channel activation). A theoretical study was also made to determine the measurable characteristics of transport fluctuations for various models of membrane pore permeation.

Project Description

Objectives: To understand the molecular mechanism of the gating conformation change by which membrane bound channels regulate ion permeation during nerve excitation. To determine the unit conductances and barrier structures of ionic channels by measurement of electric current fluctuations.

Methods Employed: Electrical noise was measured on voltage clamped nerve and muscle cells. Chick skeletal muscle cells and mouse brain neurons grown in tissue culture were transformed to more spherical shapes by application of colchicine. Electrical measurements were made using a microelectrode voltage clamp or by recording from an isolated patch of membrane via a suction electrode.

Theoretical analysis of gating kinetics and of ion transport through multibarrier pore structures were performed using stochastic differential equation models.

Major Findings: The noise studies of the postsynaptic channel in tissue cultured chick skeletal muscle have shown that the unit channel conductance is unaltered by the various treatments of this denervated cell, but that the temperature dependence of the postsynaptic channel kinetics is steeper than for innervated cells. Theoretical studies have shown that a random walk of a large dipolar subunit will give a good account of the delays observed in activation of axonal channels (Cole-Moore shift). Theoretical studies have also shown how the power spectrum of transport noise varies with barrier pattern for a class of exactly soluble barrier models.

Significance to Biomedical Research and the Program of the Institute: Electrical fluctuations provide a unique means of measuring the conductances of the unit molecular channels of excitable membranes. The experimental study of tissue-cultured muscle cells has led to the development of methods for the voltage clamp of a large variety of cultured excitable cells.

Proposed Course of Project: The major experimental project at present is the attempt to record single-channel fluctuations in mouse CNS cells grown in tissue culture (in collaboration with LN/NINCDS). An experiment is also in preparation to characterize both the electrically and chemically excitable channels of barnacle giant muscle cells by noise analysis combined with conventional voltage clamp techniques. The theoretical studies of gating and transport fluctuation will be continued.

Publications:

Sachs, F. and Lecar, H.: Acetylcholine-induced current fluctuations in tissue-cultured muscle cells under voltage clamp. Biophys. J. 17: 129-143, 1977.

Lecar, H: Physical Mechanisms of Nerve Excitability. In Studies in the Natural Sciences, Vol 13: 369-398, 1977, Plenum, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02090-04 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Lipid Bilayer Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Ehrenstein	Research Physicist	LB, NINCDS
Other:	H. Lecar	Research Physicist	LB, NINCDS
	R. Holz	Guest Worker	BB, NICHD

COOPERATING UNITS (if any)
R. Latorre, Asst. Prof., Univ. of Chicago

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to incorporate ionic channels in lipid bilayers and to study their properties - particularly their electrical properties. Most of our studies have been designed to determine the mechanisms responsible for negative resistance behavior in axons. A review of the work of several laboratories including our own, in this direction has been published. We have also attempted to incorporate biological channels into lipid bilayers by means of membrane fusion. This aspect is designed to determine single-channel properties of biological channels as well as to learn more about membrane fusion.

Project Description

The negative-resistance behavior of axons is now known to be caused by voltage-dependent changes in ionic channels incorporated in the axolemma. Comparative study of this phenomenon has been made in several different channels where discrete changes of a single channel could be observed. A wide range of behavior has been found. For example, EIM (excitability-inducing material of bacterial origin) has only two discrete conductance levels in some lipids, alamethicin has about six discrete levels, and hemocyanin has at least five discrete conductance levels and a large number of sublevels within each of the main levels. Transitions between sublevels are about three orders of magnitude faster than transitions between main levels, and special methods were required to observe them.

The information about channels in lipid bilayers provide useful models for understanding the gating mechanism in axonal channels. An indication of relevance is similarity of the magnitude of discrete conductance jumps. Channels in bilayers range from about 1 pS to about 400 pS, according to direct measurement, while indirect measurements of axon sodium channels give a value of about 10 pS.

We have attempted membrane fusion between lipid bilayers and chromaffin granules, which are storage vesicles for adrenergic transmitters. After granules were added to the solution surrounding a lipid bilayer, a discrete conductance change occurred. The magnitude of this conductance change was about 300 pS. Future plans are to optimize the conditions leading to the discrete conductance changes, and then to determine whether they are caused by fusion. Another goal is to characterize the selectivity properties of the specific pathway responsible for the enhanced ionic conductance.

Publications:

Ehrenstein, G. and Lecar, H.: Electrically gated ionic channels in lipid bilayers. Q. Rev. Biophys. 10: 1-34, 1977.

	NOTICE OF **INTRAMURAL RESEARCH PROJECT**	Z01 NS 02091-04 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mathematical Modeling

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. FitzHugh	Research Physicist	LB NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mathematical models for the following phenomena were studied:

1) The mathematical basis of periodic bursts of impulses in a simple model of a bursting pacemaker.
2) The elastic properties of a cylindrical membrane under large stretch, applied to sarcolemma and perineurium.
3) The kinetics of selective inhibition of sodium channels by drugs.

PHS-6040
(Rev. 10-76)

Project Description

The simplest qualitative model of a bursting pacemaker, which adds one slow recovery variable to the BVP (cubic) model of the nerve membrane, produces irregular patterns of bursting, which are characteristic of neurons in poor condition. The mathematical properties of this model are however of wider interest, since such irregularities, which mimic randomness in a non-random system, resemble the "chaotic" behavior seen in simpler iterative systems. The way that trajectories in the 3-dimensional phase space repeatedly pierce a certain 2-manifold, to converge to a stable but irregular limit cycle, is under investigation. These equations may have significance to the analysis of more general control systems.

The previously proposed equations (with S. Rapoport) for the stretched non-isotropic elastic membrane are physically valid only for infinitesimal deformations, while experiments have been done with large deformations. Current research on the theory of large deformations in membranes is being reviewed with the hope of producing a better model for the sarcolemma and perineurium.

A kinetic scheme which mimics the Hodgkin-Huxley sodium channel has been generalized to include the effect of a drug (yohimbine) which attaches or deättaches only at an open channel, but not at a closed one. The resulting system of sixteen linear differential equations could be solved numerically, but a simpler analytic approximation using a perturbation method, appropriate to a relatively weak drug effect, is being explored.

Publications:

FitzHugh, R.: A model of optimal voluntary muscular control. J. Math. Biol. In press.

PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 NS 02218-02 LB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Voltage-Dependent Ionic Conductance in Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. L. Gilbert	Research Physiologist	LB NINCDS
Other	W. A. Catterall	Staff Fellow	LBG NHLI
	G. Ehrenstein	Research Physicist	LB NINCDS
	Li-Yen Huang	Guest Worker	LB NINCDS

COOPERATING UNITS (if any)

J. Henderson, Medical Officer, National Naval Medical Center
R. J. Lipicky, Professor, University of Cincinnati, Ohio

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
IRP, NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

One goal of this project is to better understand the mechanisms of the Ionic conductances in membranes which are voltage dependent, i.e., excitable. Another goal is to determine how drugs influence these channels.

Recent experiments using the squid giant axon have shown that the drug, yohimbine, causes a reduction in sodium current which is reduced further when a depolarizing pulse train is applied. Studies have been made of the time course of the sodium current during the pulses and of the time course of the reduction in amplitude. The data can be fit by a model wherein yohimbine binds preferentially to open sodium channels and results in a slower rate of activation.

Other studies are involved with the effects of oxygen pressure on the giant axon of *Myxicola* as well as the effects of calcium blockers on the barnacle muscle membrane.

roject Description:

Objectives.

The primary means of determining mechanisms by which ionic channels espond to membrane potential is to change the response of the channels by ltering pressure or by applying appropriate drugs. Particular interest as been given to drugs which act to keep channels open or to keep them losed.

Methods Employed.

The measurement of ionic currents through the channels is effected by eans of voltage clamping squid axons or barnacle muscle fibers and also y means of radioisotopic measurement of ionic flux in tissue cultured euroblastoma cells.

Major Findings.

During the past year, our major emphasis was on the drug yohimbine. We found that this drug causes use-dependent inhibition of sodium channels in squid axons and that it is a competitive inhibitor of the channel-opener drug, batrachotoxin, in neuroblastoma cells.

An important question of mechanism for yohimbine blockage is whether the blockage occurs by occlusion or by an alteration in the channel-gating mechanism. The competitive inhibition with a channel-opener is a strong argument for interaction with the channel-gating mechanism. Further evidence is provided by our detailed kinetic measurements of the sodium currents in the squid axon.

Future plans include finding other use-dependent channel-closer drugs where saturating doses can be used. This would allow a more direct test of the mechanism described above.

Publications:

Ehrenstein, G.: Ion channels in nerve membranes. Physics Today (No. 10), pp. 33-39, 1976.

Henderson, J.V., Lowenhaupt, M.T., and Gilbert, D.L.: Helium pressure alteration of function in squid giant synapse. Undersea Biomed. Res. 4: 19-26, 1977.

Howell, B.J. and Gilbert, D.L.: pH-Temperature dependence of the hemolymph of the squid, *Loligo pealei*. Comp. Biochem. Physiol. 55A: 287-289, 1976.

INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Structure and function of the perineurium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.E. Taylor	Research Physiologist	LB NINCDS
Other:	S.I. Rapoport	Medical Officer, Researcher	LNP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine how and to what extent the perineurium is involved in the maintenance and regulation of the ionic and metabolic environment of the axons of peripheral nerves. The topics of interest include: (1) passive permeability to electrolytes and non-electrolytes; (2) active transport of ions; (3) facilitated diffusion or transport of amino acids and glucose; (4) electrical impedance and short circuit currents; (5) the determination of the normally existing composition of the extracellular fluid in the endoneurium.

PHS-6040
(Rev. 10-76)

Project Description:

The extracellular space in the endoneurium of peripheral nerve is isolated by the endothelial lining of cell capillaries and by the single layer of cells in the perineurium which are connected together by tight junctions. This project is concerned with the study of the role of the epithelial cell layer in the perineurium.

The methods employed are principally the standard techniques used to study unidirectional fluxes of various substances across the isolated and perfused perineural sheath of the frog or toad, including the use of radioactive tracers. In addition, histological techniques are employed in collaboration with specialists in electron microscopy, and electrical measurements are made using internal and external voltage and current supplying electrodes.

Preliminary experiments were successfully completed for the measurement of the permeability of the perineurium to nonelectrolytes and water using carbon 14 and tritium labels. Work has been in progress for measuring low frequency impedance.

The blood-nerve barrier bears the same relation to peripheral nerve axons as the blood-brain barrier does to the brain. Very little work has been done in this area. It is not known, for example, how the composition of the fluid in the extracellular space compares with cerebrospinal fluid. It would appear that the barriers in the perineurium would be intimately involved in a number of functions of the axons and in a variety of peripheral neuropathies.

The proposed course of this project is to begin to explore the passive permeability, active transport, facilitated diffusion and electrical impedance of the perineurium and to relate these to structural features as revealed by electron microscopy.

Annual Report

July 1, 1976 through September 30, 1977

Laboratory of Experimental Neurology, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

William F. Caveness, M. D.

General Statement

This is a small, inadequately staffed, laboratory that has been limited to four permanent positions. Its productivity is dependent upon the conceptual and technical competence of each of its staff, the effective contract support for processing anatomical specimen and supply of newborn monkeys, and the collaboration of scientists at Harvard University, and at other branches and laboratories at NIH.

The Laboratory was established within the Intramural Program, NINCDS, NIH, April 1969 with the physical transfer of records and equipment from New York to Bethesda in March 1970.

Current Scientific Effort:

1) Experimental Focal Seizures in the Monkey. (a) Subcortical Factors:

Blood Flow Studies: A logical extension of preceding observations is a search for the subcortical circuits and nuclear masses that are involved in the propagation of the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us it seemed wise to gain a global appraisal prior to the definitive study of individual elements. For this we have employed (^{14}C) antipyrine technique for the determination of regional cerebral blood flow during selected phases in the propagation. The assumption, based on sound observations by others, is that an increase in neuronal activity will be reflected by an increase in blood flow.

With the cooperation of the Laboratory of Cerebral Metabolism, NIMH, we have developed the (^{14}C) antipyrine method and added modifications that permit the sectioning of the whole head for the autoradiograms and the magnification by five of the radiograms for optical density measurements. This prevents distortion in the brain sections and facilities densitometric measurement of the isotope uptake in small subcortical nuclear masses, respectively. In brief: The diffusable radioactive tracer, 200 μc/Kg body weight is infused over a sixty second period during the development of a focal seizure. The monkey is then decapitated and the head immediately immersed in a Freon bath chilled to -100° C by liquid nitrogen. In short the fit is frozen in the brain. Subsequently the brain, in its case, is serially sectioned at 30 micra with a PMV Cryo-microtome temperature of -20° C. Approximately 100 sections from each brain are dried at 60° C and placed on blue sensitive X-ray film for macroautoradiographs. From the determination of the concentration of the (^{14}C) antipyrine in the arterial blood and the concentration of the tracer in the macroautoradiographs, the value of the actual regional blood flow in millilitres per gram per minute is calculated from the formula devised by Kety.

Two advantages in exploring subcortical mechanisms in the newborn are being exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have a clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. In the current series, the seizure activity is being interrupted at two, three and five minutes following the injection of penicillin. In those with the least progression, the electroencephalographic paroxysmal activity has been sporadic and limited to the right sensori-motor area. The accompanying increased blood flow has been limited to the right sensori-motor cortex and the right putamen. With the better defined electrographic activity and contralateral clinical phenomena, e.g. face or hand, the ipsilateral increase in blood flow was found in the sensori-motor cortex, supplementary cortex, putamen, globus pallidus, VL, VPL, VPM, substantia nigra and contralateral lobulus simplex of the cerebellum. With further progression of the electrographic expression and contralateral clinical phenomena involving more than one part, e.g. face and hand, the increased blood flow was found in the just described areas, plus the ipsilateral Subthalamic nucleus, Red nucleus, Pontine nucleus, and the contralateral anterior and posterior lobes and nuclei of the cerebellum.

A similar pattern with a faster progression to subcortical structures was found in pubescent and young adult monkeys.

The yield from this appraisal of brain function during the propagation of focal seizure prompted a refinement that provides a greater resolution in the autoradiograms and a closer approximation to the metabolic state, i.e. the use of [^{14}C] deoxyglucose for the quantitative estimation of the rates of glucose consumption in the various structural components of the brain. This has now been employed in five newborn and five 24 month old monkeys.

Biogenic Amines: Within the past year and one half we have added an important parameter to the studies of cortical and subcortical structures that are implicated in the propagation of focal seizures. With the Laboratory of Clinical Sciences, Section on Histopharmacology, NIMN, we are examining the changes in neurotransmitters or their enzymes in the areas that have shown increased blood flow. In the routine sectioning of the frozen head, when a structure of interest is exposed, first the section for the autoradiogram is taken, then a sample of the structure is obtained with an 18-22 gauge needle from the block remaining in the cryostat. The material is immediately transferred to the appropriate solutions for micro-assay of dopamine, norepinephrine, choline acetyl transferase, and glutamic acid decarboxylase. A small numerical ticket marks the defec in the block which is then photographed. These procedures are carried out faster than they can be described with minimal elevation in cryostat temperature. The initial findings in five newborn and five 24 month old monkeys may be summarized as follows. In the newborn the systems presumably imperfectly developed, react not at all to the fit production. In the 24 month old group there is a moderate bilateral increase in dopamin and 8 fold increase in GABA.

Electrographic Observations: These studies followed the observed prominence of the increased blood flow in the cerebellum during seizure activity. The simultaneous recording of the electrical activity from the right and left cerebral cortex and the right and left anterior lobes of the cerebellar cortex in six 3 Kg animals has demonstrated concomitant paroxysmal activity in the face area of the cerebral motor cortex and the somatotopic representation of the face in the anterior lobe of the contralateral cerebella cortex. Further, with the advance of the seizure activity to the hand area in the cerebr cortex there is similar advance to the hand representation in the cerebellar cortex. These electrical phenomena are accompanied by clonic movements in the face and hand, respectively. We plan to exploit this model in two ways: a) the determination of the effect on the cerebellar activity of interrupting the sensory input from muscles and tendo during the focal seizure, by paralyzing the animal prior to the penicillin injection, and b) determining the effect on the propagation of the focal seizure by cooling the paramedian region of the contralateral anterior lobe of the cerebellum.

Significance to Bio-Medical Research and the Program of the Institute: This established model of focal seizures will permit the manipulation of cortical and subcortical activity by pharmacological or physical agents. This should provide new approaches to medical and/or surgical therapy for selected forms of the Epilepsies.

2) Delayed Effects of Ionizing Irradiation on the Brain of the Monkey.

Three modes of brain irradiation in the young adult Macaca mulatta have been employed to demonstrate phenomena of clinical interest.

(a) In the first model, the right occipital lobe of 24 animals were exposed to 3,500 rads of orthovoltage radiation, in a single dose. The source was a General Electr Maxitron X-ray machine, operating at 250 KVP and 30 MA. The target to skin distance was 23 cm and the dose rate was 300-350 rad/min. An 0.5 copper filter and an aluminu parabolic filter resulted in an x-ray beam having a half-value layer equal to 2.15 mm of copper.

The principles demonstrated by this first mode of irradiation, i.e. a portion of the brain with a single dose of 3,500 rads, are: 1) There is a protracted delay, in week or months, before there is an overt breakdown of neuronal tissue. 2) Once this starts the destructive process proceeds quickly. 3) A prominent feature is a break in the blooc brain barrier, that not only contributes to the local destruction, but through the migrating plasmatic fluid can damage remote areas, either directly through separation and

/y

compression of neuronal elements, or indirectly through distortion of brain structures and impairment of CSF circulation. 4) The lesions seldom, if ever, heal, in the time span studied. In those instances in which the reparative processes "seal" off the lesions, there may remain focal, and perhaps diffuse, impairment in neuronal function as a remnant of the destructive processes.

At the current time this model is being exploited in the study of the Role of Anatomical and Functional Pathways in the Spread of Vasogenic Edema.

b) In the second model the whole brain was subjected to a single exposure of supervoltage irradiation. Three groups of four monkeys each received 1,000, 1,500 and 2,000 rads respectively. The source was a linear accelerator with an electron beam energy of 20 MeV, that after collimation was converted to photons by tantalum foils and flattened by a lead disc to provide a nearly constant radiation field. The dose rate was 200 rads/min. The source axis distance was 180 cm.

The principles to be derived from this second model are straightforward: 1) Whole brain exposure to 1,000 rads in a single dose causes no lesions in the first 104 weeks following irradiation. 2) Exposure to 1,500 rads causes small focal areas of necrosis in the forebrain white matter at 26 weeks, but a much more extensive involvement at and beyond 52 weeks that includes confluent areas of necrosis in grey and white matter, with focal lesions extending into the brain stem. At and beyond 52 weeks there is ventricular dilation, evidently from brain loss. 3) Exposure to 2,000 rads causes such a wide scatter of focal areas of necrosis throughout the brain, including a large number in the brain stem, that survival beyond 20-26 weeks is not possible. All showed atrophic gyri and enlarged ventricular systems.

(c) In the third model, the whole brain was subjected to fractionated exposures of supervoltage irradiation, at approximately 200 rads a day five days a week, with the same equipment described for the previous series. To bracket the human exposure of 6,000 rads, now used for malignant gliomas in several medical centers in the United States, three groups of four monkeys each received 4,000, 6,000 and 8,000 rads in courses of 4, 6, and 8 weeks, respectively.

From this third model, employing fractionated exposure to high energy irradiation, the following implications may be considered: 1) The extent of the delayed effects is dose dependent. The effects from 4,000 rads are negligible; from 6,000 rads, considerable; from 8,000 rads, profound. 2) The resultant necrotic lesions from 6,000 rads are small, widely scattered, with a predilection for the forebrain white matter but not excluding the central grey matter and brain stem. The possibility of focal as well as general clinical phenomena is inherent in this kind of brain damage. 3) The time course is important. Not only is there a delay of weeks or months before there is overt tissue breakdown but the individual lesions, evidently begin their cycle at different times, with the probability of their overall influence extending over a protracted period. Although there is an apparent trend toward recession in numbers of fresh necrotic lesions, adequate repair may be delayed or never occur. The effects at one year from 6,000 rads include considerable mineralization of the necrotic lesions, but a far greater extent of telangiectasia than found at six months. 4) At one phase in the developing areas of necrosis there may be multiple minute breaks in the blood brain barrier that when sufficient in number cause diffuse brain swelling, reflected by papilledema. 5) The lesions from 8,000 rads at six months are not strikingly different from those following 6,000 rads, however, by twelve months the extensive and coalescing lesions from the former produce gross brain destriction.

Current and future activities: A definitive series was started in July 1975 that will more closely simulate the human therapeutic experience and provide more precise information regarding the pathogenesis of the radiation lesions. Twenty-one adult male monkeys, 8 to 12 kilos in weight, were selected to more closely approximate in their age the adult humans that are irradiated for malignant gliomias. The brains of these animals were exposed to 6,000 rads of supervoltage radiation in a six week course, with alternate sides of the head exposed on alternate days. They are being killed, in sets of three, at sequential intervals following the beginning of the radiation course, to gain a better concept of the development and degree of recession of the lesions produced by this mode of irradiation over a two year period.

Significance to Bio-Medical Research and the Program of the Institute: The principal advantage in simulating a therapeutic radiation regime in a monkey model is to observe the effects in a brain uncomplicated pre-existing pathology, e.g. that resulting from a neoplasm, surgical trauma or chemotherapy. This kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurological findings following therapy. There is a direct relevance to a major program of the Cancer Institute, i.e. the Brain Tumor Study Group, and we are privileged to share in their findings as this group is made aware of our results.

3) Structural and Functional Sequelae of Penetrating Head Injury, Phase I.

A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the U. S. Navy and was implemented with the cooperation of the Surgeons General of the U. S. Army and U. S. Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from field surgeons, 1967 to 1970, was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 21.6 years. A rigorous appraisal demonstrated the uniformity and completeness of 1,540 head injury forms. Ninety per cent of these were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. Sixty per cent of those were accompanied by immediate loss in consciousness, by history. Thirty per cent were in coma responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without alteration in consciousness. These categories constitute a background against which the natural history of sequelae may be determined.

A contract project entitled, "Structural and Functional Sequelae of Penetrating Head Injury, Phase I", was submitted to NINCDS in May 1975. A Feasibility Study for Phase I was approved in March 1976, and implemented in July 1976. The conduct of this plan will update the clinical data on 1,500 selected cases from the Registry, by a review of the accumulated Military and Veteran Administration hospital records since the time of injury. The assembly of the records, abstracting the pertinent data, coding, transfer to magnetic tape, and analyses of sequelae, so identified, will be carried out in cooperation with the Medical Follow-up Agency of the National Research Council, NAS, the Physical Sciences Laboratory of the Division of Computer Research and Technology, NIH, and three specially qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School and Harvard Medical School, respectively. Concomitant with the completion of Phase I will be the planning of Phase II, i.e., and in-hospital examination of three quarters of these head injuried casualties with techniques not previously available, including computerized axial tomography for the determination of brain loss. A second project proposal will be required for Phase II.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma in man. The recent development of new techniques for evaluating functional deficits, e.g. regarding language and memory and new techniques for determining alterations in brain structure e.g. the CAT Scan, will afford an unique opportunity in Phase II for fresh insight into posttraumatic sequelae and into the function of the remaining brain.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02158-03 LEN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Subcortical Factors in Experimental Focal Seizures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. F. Caveness	Chief, Lab. of Exp. Neurology	LEN NINCDS
OTHER:	M. Kato	Visiting Scientist	LEN NINCDS
	S. Hosokawa	Visiting Fellow	LEN NINCDS
	D.M. Jacobowitz	Chief, Sect. on Histopharmacology	LCS NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Sciences, Section on Histopharmacology, NIMH

LAB/BRANCH
Laboratory of Experimental Neurology

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.8	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Focal seizures are created in monkeys, at birth and at pubescence, by injecting 25,000 units of penicillin into the right face-hand area of the cerebral motor cortex. The development and propagation of the seizure is monitored by electroencephalography, electromyography and clinical observations. At selected intervals during the propagation of the focal seizures the animal is decapitated, the head frozen and the active subcortical neuronal aggregates are delineated by an increase in the regional cerebral blood flow as determined by the (^{14}C) antipyrine method or regional cerebral glucose utilization as determined by the $[^{14}C]$ deoxyglucose method. The structures so identified are sampled for microassay of neurotransmitters or their enzymes.

Project Description

Objectives: To better understand the subcortical neuronal aggregates that are critical to the propagation of focal paroxysmal activity originating in the sensori-motor cortex.

Background: In the established animal model, focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution 3 mm into the face-hand area of the right motor cortex of the Macaca mulatta. The onset and elaboration of the attack pattern is simultaneously monitored by electroencephalography or electrocorticography, electromyography and clinical observations.

Previous oncogenetic observations stemmed from the postulate that while the cerebral cortex in the newborn is able to support focal paroxysmal activity, it is dependent upon the subcortical somatotopically linked nuclear masses and circuits for the full development of focal abnormality, its propagation to other parts of the cortex and to a common final pathway that results in the clinical seizure. In the 24 month old (pubescent) monkey, on the other hand, the propagation is under the control of cortical circuitry. To test this concept, two experimental procedures were carried out just prior to penicillin induced focal activity in the motor face area of the right cerebral cortex. By undercutting the site of activation, the propagation was blocked in the newborn and unimpeded at 24 months of age. Interrupting the intracortical connections by circumsecting the activation site had negligible effect in the newborn, but blocked the propagation at 24 months. These findings support the concept that the effective neuronal organization for the propagation of focal paroxysmal activity shifts from subcortical to cortical with maturation. The anatomical substrate in the cerebral cortex for the described seizure activity has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells demonstrated in Golgi preparations. At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits are well demarkated. In contrast, cortico connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development.

Current Methods: A logical extension of the preceding observations is a search for the subcortical circuits and nuclear masses that are involved in the propagation of the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us it seemed wise to gain a global appraisal prior to the definitive study of individual elements. For this we have employed the (^{14}C) antipyrine technique for the determination of regional cerebral blood flow during selected phases in the propagation. The assumption, based on sound observations by others, is that an increase in neuronal activity will be reflected by an increase in blood flow.

With the cooperation of the Laboratory of Cerebral Metabolism, NIMH, we have developed the (^{14}C) antipyrine method and added modifications that permit the sectioning of the whole head for the autoradiograms and the magnification by five of the radiograms for optical density measurements. This prevents distortion in the brain sections and facilitates densitometric measurement of the isotope uptake in small subcortical nuclear masses, respectively. In brief: The diffusable radioactive tracer, 200 µc/kg body weight, is infused over a sixty second period during the development of a focal seizure. The monkey is then decapitated and the head immediately immersed in a Freon bath chilled to -100°C by liquid nitrogen. In short the fit is frozen in the brain. Subsequently the brain, in its case, is serially sectioned at 30 micra with a PMV Cryo-microtome at a temperature of -20°C. Approximately 100 sections from each brain are dried at 60°C and placed on blue sensitive X-ray film for macoautoradiographs. From the determination of the concentration of the (^{14}C) antipyrine in the arterial blood and the concentration of the tracer in the macroautoradiographs, the value of the actual regional cerebral blood flow in millilitres per gram per minute is calculated from the formula devised by Kety.

Two advantages in exploring subcortical mechanisms in the newborn are being exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have a clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates.

Major Findings: In a series of 15 newborn monkeys the seizure activity was interrupted at two, three or five minutes following the injection of penicillin. In those with the least progression, the electroencephalographic paroxysmal activity was sporadic and limited to the right sensori-motor area. The accompanying increased blood flow was limited to the right sensori-motor cortex and the right putamen. With better defined electrographic activity and contralateral clinical phenomena, e.g. face or hand, the ipsilateral increase in blood flow was found in the sensori-motor cortex, supplementary motor cortex, putamen, globus pallidus, thalamic VL, VPL and VPM, substantia nigra and contralateral lobulus simplex of the cerebellum. With further progression of the electrographic expression and contralateral clinical phenomena involving more than one part, e.g. face and hand, the increased blood flow was found in the described areas, plus the ipsilateral, caudate, thalamic CM and MD, Subthalamic nucleus, Red nucleus, Pontine nucleus, and the contralateral anterior and posterior lobes and nuclei of the cerebellum. A similar pattern was found in pubescent and young adult monkeys.

The yield from this appraisal of brain function during the propagation of a focal seizure prompted a refinement that provides a greater resolution in the autoradiograms and a closer approximation to the metabolic state, i.e. the use of [^{14}C] deoxyglucose for the quantitative estimation of the rates of glucose consumption in the various structural components of the brain.

Within the past year and one half we have added an important parameter to the studies of cortical and subcortical structures that are implicated in the propagation of focal seizures. With the Laboratory of Clinical Sciences, Section on Histopharmacology, NIMH, we are examining the changes in neurotransmitters or their enzymes in the areas that have shown increased blood flow. In the routine sectioning of the frozen head, when a structure of interest is exposed, first the section for the autoradiogram is taken, then a sample of the structure is obtained with an 18-22 gauge needle from the block remaining in the cryostat. The material is immediately transferred to the appropriate solutions for micro-assay of dopamine, norepinephrine, GABA, choline acetyl transferase, and glutamic acid decarboxylase. A small numerical ticket marks the defect in the block which is then photographed. These procedures are carried out faster than they can be described with minimal elevation in cryostat temperature.

Major Findings:

In a series of focal seizures confined to the left face and upper extremity, the increased glucose utilization had a unilateral predominance in the motor, sensory and sup. motor Cortex, ventral caudal Putamen, ventral caudal Globus Pallidus, in both medial and lateral segments, VL, VPL, VPM, and CM of the Thalamus, and the contralateral simple, posterior and paramedian lobes of the Cerebellum. There was appreciable increase in Dopamine in the Caudate and Putamen, that is bilaterally symmetrical, there is a slight increase in the substantia nigra. There was a moderate increase in GABA noted throughout with a very marked increase in the Globus Pallidus and Substantia Nigra. This increase is bilaterally symmetrical.

A third method that is being employed is based on the observed prominence of the increased blood flow in the cerebellum during the seizure activity and consists of the simultaneous recording of the surface electrical activity from the cerebral cortex and the cerebellar cortex during the propagation of the paroxysmal phenomena.

For this fourteen monkeys, 3.0-3.5 Kg, under halothane anesthesia, received bilateral convexity and suboccipital craniectomies. The dura was reflected over the precentral gyri and slit just beneath the transverse sinus. The cerebral cortical electrodes consisted of four pairs of platinum contacts, encased in a thin plastic sheath. Each contact was 1 mm in diameter with 2 mm clearance between the inner edges of each pair. The pairs were separated by 4 mm. The total array was placed adjacent to the right central sulcus, opposite the convexity of the arcuate sulcus, providing 15 mm coverage of the motor strip, i.e. extending from the face-hand area over the representation of arm, trunk, and leg. A similar array of electrodes was slipped beneath the tentorium, providing 15 mm coverage over the left paravermal region of the cerebellum, with its somatotopic representation of face, upper and lower extremities. For ancillary use, comparable electrode arrays were positioned over the left cerebral cortex and the right cerebellar cortex, respectively. Closures were effected by gel foam and scalp clips. Three hours after the completion of the surgical procedures and the end of the anesthesia, the critical observations were conducted with the animal fully alert, in a prone position, the head fixed and the limbs restrained. The focal seizures were induced in the usual manner by the injection of 25,000 units of penicillin into area 4 of the face-hand area of the right motor cortex. Prior to and throughout the development and propagation of the seizures, bipolar recording of the surface electrical activity was obtained, from the indicated pairs of electrodes, with a Grass 8-18 electroencephalograph. Clinical manifestations were monitored by visual observations and by electromyography from the musculature of the left face and extremities. After one hour of data collection, the animal was killed with an excessive dose of pentobarbital and the electrode positions verified.

Results: There was a very close correlation between the initial and subsequent rhythmic spike activity from the right cerebral and the left cerebellar cortices, as evidenced by the following: a) In eleven out of fourteen monkeys, the time of onset of the spike activity in the right cerebral cortex and the left cerebellar cortex was identical, as recorded by the ink-writing equipment. In the remaining three, the lag in the appearance of the first spike from the contralateral cerebellum was 20", 40", and 64" respectively; b) With the cessation of spike activity in the cerebral cortex, there was a simultaneous cessation in the cerebellar spike activity; c) With progression of the focal fit, and the increase in complexity of the cerebral wave form, e.g., bursts of multiple spikes, the same complexity appeared in the cerebellar cortex; d) When the frequency of the paroxysmal activity changed in the cerebral cortex, e.g. a sudden transient shift from 3 Hz to 5 Hz, a precisely similar change took place in the cerebellar cortex; e) With the advent of clinical expression the spike activity in the electromyogram was coincident with the spike activity in both the cerebral and cerebellar cortices. The location of the spike activity was somatotopically related, i.e. the paroxysmal activity in the face-hand area of the precentral gyrus of the right cerebral cortex was coincident with that in the left lobulus simplex, representative of the face in the cerebellar cortex; f) When the paroxysmal activity progressed from the face-hand area of the cerebral cortex to those of arm, trunk, and leg, a comparable progression took place from the contralateral lobulus simplex, representing face, to those parts of the anterior lobe of the cerebellum representing the upper and lower extremities. These phenomena were accompanied by an appropriate contralateral clinical progression from face to upper and lower extremities. In three of the animals in which the penicillin injection was shifted to the leg-foot areas in the motor strip, the somatotopic progression was in the opposite direction, simultaneously in the cerebral and cerebellar cortices, as well as in the clinical expression.

Proposed Course: The energetic pursuit of the combined deoxyglucose and neurotransmitter studies of subcortical structures that are critical to the propagation of a focal seizure. Further, the cerebral-cerebellar electrographic model will be explained in two ways: a) the determination of the effect on the cerebellar activity of interrupting the sensory input from muscles and tendons during the focal seizure, by paralyzing the animal prior to the penicillin injection, and b) determining the effect on the propagation of the focal seizure by cooling the paramedian region of the contralateral anterior lobe of the cerebellum.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should delineate the principal subcortical factors in the propagation of focal seizures in the monkey and thereby provide fresh approaches to therapeutic efforts in man.

Publications:

Caveness, W.F.: Epilepsy, a product of trauma in our time. Epilepsia, 17: 207-215, 1976.

Caveness, W. F., Kosaka, K., Hosokawa, S. and O'Neill, R.R.: Cerebral-cerebellar paroxysmal activity in experimental focal seizures. Ann. Neurol. 1:287-289, 1977.

Hosokawa, S., Yamashita, Y., Ueno, H., and Caveness, W.F.: Regional cerebral blood flow pattern in subcortical propagation of focal seizures in newborn monkeys. Ann. Neurol. 1:225-234, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02190-02 LEN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Thermal Manipulation of Paroxysmal Neuronal Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. F. Caveness	Chief, Lab. of Exp. Neurology	LEN NINCDS
	M. Kato	Visiting Scientist	LEN NINCDS
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COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the study of subcortical structures that are brought into play with the propagation of paroxysmal activity from a penicillin focus in the motor cortex of the monkey, a question of importance is: which of these neuronal aggregates are essential to the full development of the experimental focal seizure? In seeking an answer, neuronal blockade of structures with previously demonstrated involvement will be brought about by a stereotactically controlled cryoprobe. The instrument at hand permits graded cooling at its tip, that is 0.1 cm in diameter and monitored by a microthermocouple. By using non destructive degrees of cooling in selected subcortical areas, the objective will be to temporarily interrupt the progression of the paroxysmal activity, thereby demonstrating the importance of these areas in fit production.

PHS-6040

Project Description

Objectives: To interrupt, in a reversible manner, the paroxysmal activity in cortical or subcortical neuronal aggregates during the propagation of an experimental focal seizure.

Background: The delineation of cortical and subcortical structures that sequentially come into play during the propagation of a focal seizure has been accomplished with the use of (^{14}C) antipyrine regional cerebral blood flow studies. It is expected the definition of the role played by these structures will be enhanced by the [^{14}C] deoxyglucose method which should provide quantitative evaluations of glucose utilization. When the latter technique is used in combination with the neurotransmitter studies, as described in Project No.: Z01 NS 02158-03 LEN, a limited number of structures should stand out as of critical importance to the production of the seizure. The structure so identified will be the target for thermal manipulation.

Methods Employed: Utilizing the established model of focal seizures induced by the injection of 25,000 units of penicillin in the face-hand area of the right motor cortex, an attempt has been made to prevent or to interrupt the propagation of the paroxysmal activity by cooling selected subcortical structures. Structures considered include the ipsilateral putamen, globus pallidus, thalamic VL, VPL or VPM, substantia nigra, and contralateral paravermal aspects of the anterior and posterior lobes of the cerebellum. The mode of locally lowering the temperature is a specifically constructed cryoprobe, 0.1 cm in diameter and 10 cm in length that may be attached to a stereotactic probe carrier for proper insertion into the monkey brain. The cooling is limited to a silver hemisphere, 0.1 x 0.05 cm, at the tip which is monitored by a microthermocouple. The tip also serves as a monopolar electrode. The graduations in control of cooling at the tip should provide reversible blockade of neural function, expected between -5° and -10° centigrade. This instrument is at hand and operational.

Major Findings: In a series of 3 animals with correctly placed cryoprobes in the ventral caudal portion of the putamen and the ventral caudal portion of the Globus Pallidus, cooling to -10° C produced no alteration in the focal seizure findings. The next targets will be thalamic nuclei.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should effectively supplement the understanding of subcortical factors in the propagation of focal seizures. Further, they should provide a fresh approach to surgical therapy for selected aspects of the Epilepsies.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02159-03 LEN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Whole Brain Irrdadiation within the Therapeutic Range

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.1	1.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As a part of a continuing series of studies on the delayed effects of ionizing irradiation on the brain of the monkey, the current effort includes sequential observations after whole brain exposure to 6000 rads of supervoltage radiation, in divided doses over a six week period. The protocol simulates as closely as possible that used by twelve medical centers in therapy of malignant gliomas in humans.

PHS-6040
(Rev. 10-76)

Project Description

Background: Previous studies of the delayed effects from 3,500 rads of orthovoltage radiation, in a single dose, to the right occipital lobe of the young adult Macaca mulatta employed three approaches that yielded the information reported in a completed project in a prior year.

In brief, the first was concerned with those structural changes that could be detected through the sequential sacrifice of 24 monkeys, in sets of three, over a period of 44 weeks. The early ultrastructural changes were found to be subtle, implicating glycogen metabolism, and perhaps enzyme systems, as reflected by altered lysosomes. With routine histology, and Golgi Cox preparations, the sequential changes prior to 20 weeks were limited to minimal, scattered astrocytic or microglial reactions with occasional perivascular collections of mononuclear cells. Around twelve weeks there were detectable though not pronounced alterations in dendritic arbors and to a less extent, a loss in cell bodies. From 20 to 24 weeks, there was a rather abrupt breakdown in neural tissue, represented mostly clearly in focal areas of myelin destruction, accompanied by proliferative and degenerative changes in astrocytes, microglia and oligodendroglia, and collections of mononuclear cells. Vascular changes included proliferation, occlusion, degeneration and hemorrhagic exudates. Around the periphery were hyperplasia of glia, telangiectasia, and an inflammatory reaction. In the following weeks the focal areas coalesced into wider areas of tissue destruction that were accompanied by reparative processes of varying but incomplete degree.

As a part of this complex lesion there was a massive break in the blood brain barrier, the clinical implications of which could be profound. To delineate this, an additional set of monkeys received by vein, Evans Blue dye, 2 ml/Kg two hours before they were killed. The irradiated right occipital lobe was swollen and deeply stained. The swelling extended from the irradiated region throughout the right hemisphere causing gross brain distortion. To monitor the changes in cerebral spinal fluid pressure, an indwelling catheter reservoir system was employed that permitted sequential measurements of cisternal pressure. In 8 out of 12 irradiated monkeys the pressure curves indicated a delayed, abrupt rise that might take place as early as 18 weeks or as late as 36 weeks following exposure. The pressure increase achieved on elevation of 3 or more times baseline within a period of two weeks and was reflected in papilledema (4/8), oculomotor palsies (3/8), behavioral blindness (4/8), gross depression in visual evoked response (8/8) and predominantly delta activity in the background electroencephalographic activity (8/8). The four animals that developed blindness are of special interest. The unilateral destruction of the visual cortex is insufficient to account for this as is the bilateral papilledema, since the blindness and papilledema did not always occur in the same animal. Rather, we believe the blindness to be the result of gross distortion of the visual pathways within the brain, i.e. the swelling in the right hemisphere and the compression in the left, implicating particularly the lateral geniculate bodies. Three of the twelve animals showed only a mild elevation of CSF pressure, never more than twice baseline, between the 20th and 36th week.

Observed for 52 weeks, none of these animals showed abnormal clinical signs, although all showed mild depression of the visual evoked response on the right. When killed, all showed some staining with Evans Blue, but less pronounced histological changes at the irradiated site than that seen in the previous group of eight monkeys. A single monkey showed an appreciable but more gradual increase in CSF pressure, three times baseline at 22 weeks with a return to, and below, baseline from the 40th week. There was papilledema from the 18th to 30th week, and absent visual evoked response from the 25th to 30th week, then a gradual recovery of the latter during the remainder of the 52 weeks of observation. When killed, the brain showed no staining with Evans Blue but rather a shrunken fibrotic lesion in the irradiated right occipital lobe. The lateral geniculate body on the right showed cell loss involving all lamina, evidently the remnant of pronounced edema in the right hemisphere.

As an extension to this model we determined the regional cerebral blood flow (rCBF), by the (^{14}C) antipyrine method for evidence of metabolic impairment, not only within the developing lesions, but throughout the brain. For this, one monkey was killed at baseline, three during the phase of development of increased CSF pressure, one at its peak, and one upon recovery. Two unirradiated animals served as controls. The findings indicated that as the CSF pressure increased the blood flow decreased. This decrease was at first greater in the right hemisphere, the side of the lesion, and in the white matter more than the grey matter. However at the highest pressures the blood flow was decreased bilaterally in both white and grey matter. Of perhaps grave significance was the only partial recovery in blood flow in the monkey whose intracranial pressure had returned to normal. While there was greater improvement in grey than white matter, there remained a diffuse reduction in blood flow suggesting a long-term impairment in cellular metabolism and/or blood flow regulatory mechanisms.

After these extensive studies of the delayed effects following a single exposure of 3,500 rads of orthovoltage radiation to a limited portion of the brain, we were confronted with two questions: 1) What, if any, of these effects would be observed if the whole brain were exposed to supervoltage irradiation in a single dose, and 2) What, if any, of these effects would be observed if the exposure were divided and delivered to the whole brain a manner similar to that utilized in standardized therapeutic procedures? Of particular interest was the protocol employed by the Brain Tumor Study Group, sponsored by the Cancer Institute, NIH. For their irradiation of malignant gliomas, they use a 6,000 rads mid plane tumor dose over a period of 6-7 weeks with lateral parallel opposing fields. With megavoltage equipment, source axis distance of not less than 75 cm and a mid plane depth dose rate of not less than 33 rads per minute, daily increments of approximately 200 rads per day are delivered during a five day week.

Current Objective: To determine the effects on normal brain from super voltage radiation within the therapeutic range.

Methods Employed, first: In twelve monkeys, 3 Kg in weight, the whole brain was subjected to a single exposure of supervoltage irradiation. Three groups of four monkeys each received 1,000, 1,500 and 2,000 rads, respectively. The source was a linear accelerator with an electron beam energy of 20 MeV, that after collimation was converted to photons by tantalum foils and flattened by a lead disc to provide a nearly constant radiation field. Shielding with three to eight inches of lead provided protection except for an opening limited to the external borders of the brain, as determined by routine skull x-ray. Dosimetry by thermoluminescent dosimeters and ionizing chambers indicated a variation of less than 5% throughout phantom brains, irradiated unilaterally, from the left. The dose rate at mid plane was 200 rads per minute. The source-axis distance was 150 cm. The geometry of the exposure was determined at the Armed Forces Radiobiology Research Institute and the irradiations were carried out in that facility. Four additional monkeys served as controls. When one from each group was killed at 26, 52, 78, or 104 weeks after irradiation the brains were fixed by perfusion with paraformaldehyde, embedded whole in celloidin, and cut in serial sections at a thickness of 35 μ. Every twentieth section was stained for myelin by the Loyez method and the adjacent section for nerve cells and glial nuclei with the Nissl method. An additional set, 100 sections apart, was stained with H & E and PAS for vascular and other structures.

Major Findings: Those with the whole brain exposed to 1,000 rads in a single dose demonstrated no clinical signs. Those irradiated with 1,500 rads showed progressive neurological abnormalities, including diminished motor activity, myoclonic jerks, high amplitude slow waves in the background EEG, papilledema and loss of visual acuity. With whole brain exposure to 2,000 rads, none of the monkeys survived beyond 26 weeks. Their deteriorating condition included crippling loss in motor power and dexterity with finally an inability to injest food. Three out of the four, had pronounced papilledema beginning in the 8th week, the fourth, had blurred disc margins in the 12th week, that was less evident at the time of sacrifice.

The brains of those exposed to 1,000 rads showed no abnormality. The brains of those subjected to 1,500 rads, that were killed at or beyond 52 weeks showed evidence of swelling and symmetrically dilated ventricular systems. When sectioned, the brain of the monkey killed at 26 weeks, showed scattered focal lesions in the forebrain white matter. These consisted of discrete, minute areas of necrosis, in some of which there were mineral deposits. The monkey killed at 52 weeks, showed wide spread areas of coalescing necrosis involving primarily the white matter of the cerebral hemispheres, along with focal lesions in the brain stem and cerebellum. In the focal lesions there was some mineralizeration with very little found in the confluent areas of necrosis. Vascular changes included amorphorous, subendothelial deposits in the small arteries, and capillary telangiectasia. The monkey killed at 78 weeks, had a pattern of confluent and focal necrosis similar in kind, but less in degree than that shown in the monkey killed at 52 weeks. The monkey killed at 102 weeks, on gross examination showed atrophic as well as edematous changes, along with cerebellar tonsilar herniation. The histological examination of this brain is not yet complete. With whole brain exposure to 2,000 rads, the most striking gross abnormality in all four brains was the symmetrical enlargement of the ventricular system. Upon sectioning, they all had a wide scatter of focal necrotic lesions that, though very high in number had not yet shown coalescing or confluence. The large number of fresh lesions in the brain stem undoubtedly contributed to the inability of any of the four to survive beyond 26 weeks.

Methods Employed, second: In twelve monkeys, the whole brain was subjected to fractionated exposures of supervoltage irradiation, at approximately 200 rads a day five days a week, with the same equipment described for the previous series. To bracket the human exposure of 6,000 rads, now used for malignant gliomas in twelve centers in the United states, three groups of four monkeys each received 4,000, 6,000 and 8,000 rads in courses of 4, 6, and 8 weeks, respectively.

Major Findings: The monkeys exposed to 4,000 rads showed no functional abnormalities. Those that received 6,000 rads showed alteration in visual evoked response and/or papilledema from the 8th week following irradiation, the latter usually receding between the 16th and 24th week. From the 32nd week high amplitude slow waves were apparent in the EEG. Those that received 8,000 rads exhibited a course not unlike those that received 6,000 rads up to 26 weeks. Beyond that time there was a progressive deterioration reflected in severe impairment in motor function, papilledema followed by optic atrophy and behavioral blindness, and prominent delta activity in the EEG. The brains of the animals that received 4,000 rads showed no structural abnormalities. In those exposed to 6,000 rads, the animal killed at 26 weeks, showed 143 lesions scattered throughout the forebrain, predominantly in the white matter of the corona radiata, centrum semiovale and internal capsule, with a fair number in the central grey matter. These consisted of areas of necrosis, 1 mm or less in diameter, attended with varying degrees of vascular, glial and macrophage reactions. The individual lesions were of different ages. The most recent consisted of a central core of necrotic brain surrounded by a narrow rim of macrophage response. Slightly older lesions contained numerous macrophages throughout. The oldest showed mineral deposits that stained for both calcium and iron. Myelin sections adjacent to the Nissl sections at times revealed corresponding areas of palor that often exceeded in extent the area of necrosis, suggesting focal "vasogenic" edema. Adjacent to the punctate lesions were occasional grossly altered vascular endothelial cells. Quite apart from these, were areas of capillary proliferation, or telangiectasia, 5-8 mm in extent. At 52 weeks there were only 12 small necrotic lesions, and most of these were mineralized. In addition there were innumerable minute areas of mineral deposits that may have represented remnants of larger necrotic lesions, or degenerated vascular walls or plasma products. A more active process was reflected in widespread areas of dilated capillaries or telangiectasia, more than were seen at 26 weeks. At 66 weeks there were 63 small necrotic lesions with varying degrees of associated reactions including mineralization in 49. There were also scattered minute mineral deposits bilaterally. At 78 weeks there was a wide distribution of 242 focal necrotic lesions with a somewhat greater extension into the brain stem than was seen in the animals that were killed at shorter intervals. The accompanying vascular reactions and mineral deposits were, however, more apparent. From the preceding it appears that in the monkey exposed to 6,000 rads there is a tendency to fewer and fewer necrotic lesions to appear with the passage of time with a progressively larger number that are mineralized, suggesting a tendency toward healing. The monkey killed at 78 weeks showed more lesions than were found at earlier intervals, but few of these were recent, sustaining the impression of a trend toward repair. The effects from 8,000 rads at 26 weeks included 64 focal lesions, that were similar in kind but greater in degree, than those from 6,000 rads. One monkey killed off schedule at 32 weeks, showed 2,089 focal lesions throughout the white and grey matter of the brain, with 115 in the brain stem. At 52 weeks there was a confluence of necrotic lesions in the swollen cerebral hemispheres including thalamus and basal ganglia, but fewer focal lesions in the brain stem than were found in the monkey that was incapasitated at 32 weeks. At 78 weeks there was massive brain destruction from confluent necrotic lesions in the cerebral hemispheres. The loss in brain substance was reflected in the atrophy of the gyri and marked enlargement of the ventricular system. These monkeys exposed to 8,000 rads demonstrate with the passage of time a consistant, progressive increase in number and confluence of necrotic lesions. Even within this relentless course the monkey killed at 78 weeks showed somewhat less active and more atrophic process than the monkey killed at 52 weeks.

Present Activities: A definitive series was started in July 1975 that will more closely simulate the human therapeutic experience and provide more precise information regarding the pathogenesis of the radiation lesions. Twenty-one adult male monkeys, 8 to 12 kilos in weight, were selected to more closely approximate in their age the adult humans that are irradiated for malignant gliomias. The brains of these animals were exposed to 6,000 rads of supervoltage radiation in a six week course, with alternate sides of the head exposed on alternate days. They are being killed, in sets of three, at sequential intervals following the beginning of the radiation course, to gain a better concept of the development and degree of recession of the lesions produced by this mode of irradiation. Their clinical course is being monitored by neurological observations, funduscopic photography and spinal fluid examination for protein and lactic dehydrogenase. To these regularly scheduled examinations, computerized axial tomography was added in November 1976. The latter should not only provide evidence of brain loss as reflected in dilated ventricles but, with the use of enhancement techniques, evidence of breaks in the blood brain barrier gave a dynamic appraisal of the location and degree of tissue breakdown, and repair.

Significance to Bio-Medical Research and the Program of the Institute: The principal advantage in simulating a therapeutic regime in a monkey model is to observe the effects in a brain uncomplicated by pre-existing pathology, e.g. that resulting from a neoplasm, surgical trauma or chemotherapy. This kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurological findings following therapy. Further, there is a direct relevance to a major program of the Cancer Institute, i.e. the Brain Tumor Study Group, and we are priviledged to share in their findings as this group is made aware of our results.

Publications:

Caveness, W.F., Kemper, T.L., and O'Neill, R.R.: Delayed vasogenic edema following irradiation of the monkey brain. In Pappius, H.M., Feindel, W. (Eds) Dynamics of Brain Edema. pp. 50-56, Berlin, Heidelberg, New York, Springer Verlag, 1976.

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Kemper, T.L., O'Neill, R.R., and Caveness, W.F.: Effects of single dose supervoltage whole brain radiation in the Macaca mulatta. J. Neuropathol. Exp. Neurol. 1977 (In press)

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PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 NS 02189-02 LEN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Anatomical and Functional Sequelae of Penetrating Head Injury, Phase I

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Laboratory of Experimental Neurology

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Objective: To determine the loss in brain substance and the alteration in brain function, 8-10 years after brain damage incurred during the Vietnam war. The resource for this is a Registry of 1500 Head Injuries compiled in the field by Military Surgeons from 1967 to 1970. The characteristics of the injury and the initial disability is recorded in detail, according to a prospective plan.

To achieve this objective, a plan has been developed, titled Phase I, and a team assembled to a) collect, review and analyze the intervening medical records and b) plan, Phase II. The latter will utilize an unique approach to the loss or alteration in structure, i.e., computerized axial tomography, and other techniques, not available in previous studies of sequelae. A Feasibility Study for Phase I was initiated in the Autumn of 1976.

Project Description

Objectives: To determine the loss in brain substance and the alteration in brain function, 8-10 years after brain damage incurred during the Vietnam War.

Background: A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the U. S. Navy and was implemented with the cooperation of the Surgeons General of the U. S. Army and U.S. Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from field surgeons, 1967 to 1970, was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 21.6 years. A rigorous appraisal demonstrated the uniformity and completeness of 1,540 head injury forms. Ninety per cent of these were the result of explosively propelled missiles. 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. Sixty per cent of these were accompanied by immediate loss in consciousness, by history. Thirty per cent were in coma responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without alteration in consciousness. These categories constitute a background against which the natural history of sequelae may be determined.

Current Methods: A contract project entitled, "Anatomical and Functional Sequelae of Penetrating Head Injury, Phase I", was submitted to NINCDS in May 1975. A Feasibility Study for Phase I was approved in March 1976, and was implemented in September 1976. The conduct of this plan will update the clinical data on 1,500 selected cases from the Registry, by a review of the accumulated Military and Veteran Administration hospital records since the time of injury. The assembly of the records, abstracting the pertinent data, coding, transfer to magnetic tape, and analyses of sequelae, so identified, will be carried out in cooperation with the Medical Follow-up Agency of the National Research Council, NAS, the Physical Sciences Laboratory of the Division of Computer Research and Technology, NIH, and three qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School and Harvard Medical School, respectively. Concomitant with the completion of Phase I will be the planning of Phase II, i.e., an in-hospital examination of three quarters of these head injured casualties with techniques not previously available, including computerized axial tomography for the determination of brain loss. A second project proposal will be required for Phase II.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma in man. The recent development of new techniques for evaluating functional deficits, e.g. regarding language and memory and new technicques for determining alterations in brain structure e.g. the CAT Scan, will afford an unique opportunity in Phase II for fresh insight in posttraumatic sequelae and into the function of the remaining brain.

This project is consistent with the efforts to improve the understanding and management of the effects of trauma now being sponsored by the Stroke and Trauma Program of NINCDS and the General Trauma Program of the Institute of General Medical Sciences.

The participation of the Medical Follow-up Agency, National Research Council, and the professionals at the Universities indicated are made possible through contract mechanisms.

ANNUAL REPORT
July 1, 1976 through September 30, 1977
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological and Communicative
Disorders and Stroke
Janet V. Passonneau, Chief, Acting

The Laboratory of Neurochemistry is composed of three sections, the Section on Cellular Neurochemistry, the Section on Enzymes, and the Section on Neuronal Development and Regeneration. These sections are engaged in a variety of projects.

Section on Cellular Neurochemistry

The program on ischemic-induced changes in brain metabolites, neurotransmitters, and cyclic nucleotides will be continued. The investigations will include the study of drug effects on ischemic changes in the cerebral cortex and on the recovery when circulation is reestablished after an ischemic episode. Preliminary results have indicated that non-anesthetic doses of thioamlal increased the survival rate from 10 to 70% (6 days after a 20 minute ischemic period).

The effect of oxygen and glucose deprivation in C-6 astrocytoma and C-1300 neuroblastoma cells in culture is being investigated as a corollary to the ischemic studies in gerbils.

The facilities have now been set up to do micro-dissection and microanalysis on frozen dried sections weighing from 0.1-1.0 μg. The effects of ischemia on different regions in the brain, and on cortical layers will be examined.

Another project has been concerned with the effects of seizures induced either by electroshock or drugs (isoniazid and pentylenetetrazole) on γ-amino butyric acid (GABA) and cyclic GMP in the cerebral cortex and cerebellum of mouse brain. One group of anticonvulsant drugs, such as dipropylacetic acid, increased GABA and decreased cyclic GMP in the cerebellum. Another group of drugs, including phenobarbital and phenytoin decreased the cyclic GMP but had no effect on the GABA in the cerebellum. Neither group had a significant effect on the cyclic GMP in the cerebral cortex. Those agents which affected the GABA levels had similar effects in both the cerebral cortex and cerebellum. During the period of increased GABA and decreased cyclic GMP concentrations, the threshold for seizure as determined by electroconvulsive shock was increased.

Convulsants such as isoniazid and pentylenetetrazol increased the cyclic GMP in both cerebellum and cerebral cortex. None of the pharmacological manipulations tested significantly affected the levels of cyclic AMP. Maximal electroshock increase both cyclic AMP and cyclic GMP in the cerebral cortex and cerebellum. Pretreatment of the mice with 20 mg/kg phenytoin, a dose which diminishes the convulsive response, reduced the cerebellar cyclic GMP response by 50 percent but had little or no effect on the other cyclic nucleotide changes.

The studies of the effects of convulsant and anti-convulsant agents on cerebellar and cerebral metabolites, putative neurotransmitters, and cyclic nucleotides will be continued. Substantial progress has been made in the investigation of maximal electroshock in cerebellar layers, from frozen dried sections. We intend to extend these studies to the deeper nuclei, and larger cell bodies (Purkinje cells). For the latter a new balance will be constructed with a sensitivity of 2 ± 0.02 ngrams.

The GABA shunt metabolites and enzymes are also being examined in C-6 astrocytoma and C-1300 neuroblastoma cells in culture. GABA in neuroblastoma cells is found to be one-tenth the concentration found in glioma cells, while GABA transaminase activity is either lacking in astrocytoma cells, or is less than one-tenth of the activity in neuroblastoma cells. The cells afford an opportunity to compare the effects of anticonvulsants and inhibitors of the GABA pathway on cells of glial and neural origin.

Methodology is being worked out to study the retinal layers in frozen dried material. Again we will study the localization of metabolites, putative neurotransmitters, cyclic nucleotides and enzymes. There has been a substantial body of work on the glycolytic pathway in retina using these microtechniques but very little on the Krebs cycle intermediates. Because of the layered structure of the retina, and the concentration of mitrochondria in the inner segment (layer 2b) the question of sequestration of certain metabolites of the citric acid cycle in these organelles may be approached. While there have been many investigations of the cyclic nucleotide systems in retina, these studies have been largely in outer rods separated from fresh retina by centrifugation. We will be able to localize the cyclic nucleotides in material fixed by freezing or microwave irradiation, procedures which are more likely to preserve *in vivo* conditions. Once base lines are established, the effects of light, anoxia and retinopathies will be examined.

Section on Neuronal Development and Regeneration

The Section on Neuronal Development and Regeneration is attempting to use transplantation techniques to elucidate factors involved in neuronal (1) nerve fiber regeneration, (2) tropic nerve function, and (3) selectivity and plasticity in forming neural connections. The purpose of one project is to investigate the possible use of a peripheral nerve allograft (i.e., a graft between genetically different animals of the same species) to provide a neurilemmal system (i.e., Schwann cells and their basement membranes) which might promote the regeneration of host nerve fibers at sites of injury to the peripheral or central nervous system. Presently studies are being performed in rats to determine whether nerve allografts are antigenic (i.e., able to incite an immune response and are destroyed by it), and whether tissue-typing has any influence on nerve allograft survival. The success of nerve allografts in the repair of the injured peripheral nervous system will ultimately be revealed by determining whether nerve allografts permit host motor or sensory nerve fibers to regenerate through them and subsequently reinnervate muscle or taste buds. In the central nervous system the nerve allograft will be used to try to encourage central fibers to restore hemidiaphragmatic respiratory function which was previously paralyzed by hemisection of the cervical spinal cord. Most of the above cited studies are in progress and only initial results on nerve graft antigenicity are available. These results show that nerve allografts do incite an immune response and that grafts exchanged between animals exhibiting major histoincompatibility are rejected within five weeks whereas grafts exhibiting other histoincompatibilites still survive. Other results have also shown that immunological rejection occurs in allografts in the spinal cord--a transplantation site heretofore regarded as immunologically privileged. Because rejection of nerve allografts can occur it will now be necessary to evaluate these grafts under conditions of immunosuppression. Consequently, studies are being performed with nerve allografts made to immunologically tolerant hosts (i.e., animals made unresponsive to an allograft because suppressor lymphocytes or blocking antibodies were induced in them by their neonatal exposure to donor allograft antigens). Finally, if allografts fail in the central nervous system, the immunlogically deficient nude mouse will be used to see if its injured spinal cord can be repaired by the cells or cell products of a xenograft (i.e., a graft from a different species of animals) of spinal cord taken from those amphibians or reptiles which readily reconstitute their injured nervous tissue.

The immunologically deficient nude mouse is also being utilized to study cross-species trophic nerve function. Such studies are possible because a congenital absence of the thymus gland in the nude mouse prevents its T-lymphocytes from maturing and becoming competent to reject an allograft, xenograft, or a combination of an allograft and xenograft. It has already been shown, for example, that neurons in a xenograft of rat sensory ganglion can survive after transplantation to the anterior chamber of the eye of the nude mouse and that these rat neurons can regenerate nerve fibers into a cotransplanted allograft of mouse tongue tissue and induce the formation of taste buds. Since taste buds are not readily grown in tissue culture, the transplantation of grafts to the nude mouse presents a way to determine whether various animals have similar or different neuronal and epithelial cell recognition molecules involved in taste bud development. Furthermore, it should be possible to analyze pathological tissues for possible trophic defects. For instance, in the human disease familial dysautonomia, normal and dysautonomics. Preliminary results have also indicated that cross-species neuromuscular innervation can occur in the nude mouse. In that study an intact muscle from a rat was transplanted into the leg of a nude mouse (i.e., into the site formerly occupied by the excised extensor digitorum longus muscle of the nude mouse) where it survived and became reinnervated by the nude mouse's motor nerve fibers. Furthermore it seems that "in vivo", unlike "in vitro", cross-species neuromuscular innervation causes the differentiation of histochemically mature muscle fibers. The grafting of animal and human muscle to the nude mouse could help elucidate whether certain neuromuscular disorders in these species are neurogenic or myogenic in etiology.

Section on Enzyme Chemistry

The Section on Enzyme Chemistry has four current projects. The first deals with the enzymological aspects of neural function. Current studies are designed to elucidate the structure, function and regulatory mechanisms of the active transport system for Na^+ and K^+ in excitable membranes. Immunochemical studies have shown that the glycoprotein subunit of the (Na^+ and K^+)-ATPase is closely associated with ouabain-binding site of the enzyme. Transient kinetic studies have provided new and more direct evidence that the conformation of the transport ATPase is dependent upon the occupation of Na^+ - and K^+ -binding sites in addition to nucleotide binding and phosphorylation.

An investigation of ouabain-sensitive cation fluxes in erythrocytes from subjects with myotonic dystrophy has found normal ratios of Na^+ flux to K^+ flux in contrast to a recently published report. Studies have been initiated in an effort to characterize endogenous tissue constituents that interact with the ouabain-receptor of the $(Na^+ + K^+)$; ATPase.

A second project is concerned with the localization of $(Na^+ + K^+)$-ATPase in the nervous system. Electron microscopic localization of the $(Na^+ + K^+)$-ATPase in brain of the knife-fish has continued in collaboration with the Department of Anatomy, University of Tennessee Medical School, Memphis, Tennessee. More recent studies have centered upon the localization in certain astrocytic processes and in sub-lemmal cisternae of axons.

In collaboration with the Department of Anatomy, Harvard University Medical School, Boston, Massachusetts, we are exploring the use of E.M. techniques to establish the "sidedness" of the localization of ATPase antigens in electoplax membranes.

A third project is the study of the physiological and biochemical correlates of lipid metabolism in brain. It is the purpose of this study to resolve the cold resistant nature of the brain of a mammalian hibernator. The topics of interest and investigation are: (1) Fatty acid metabolism, primarily that associated with the desaturation of fatty acids, (2) glycerophospholipid metabolism, (3) membrane lipid turnover, and (4) membrane biogenesis. The use of the hibernating species of mammal, the hamster, is employed in these studies since it can alter naturally when cold exposed, both the chemical and physical nature of its neural elements.

A fourth project is the study of GABA and nucleotide metabolism in brain mitochondria. The objective of these studies is to determine the interrelationships between substrates for brain mitochondria and the turn-over of mitochondrial nucleotides. In particular, we are exploring a possible regulatory role of γ-aminobutyrate in mitochondrial energy metabolism. We have established that substrate level phosphorylation has a special role rephosphorylating adenylic acid in brain as well as liver. Methodology and baseline values for the levels and turnover rates of brain mitochondrial nucleotide have been determined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00813-16 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Enzymological Aspects of Neural Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	D. H. Jean, Ph.D., Visiting Associate	LNC	NINCDS
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.8	3	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Current studies are designed to elucidate the structure, function and regulatory mechanisms of the active transport system for Na^+ and K^+ in excitable membranes. Immunochemical studies have shown that the glycoprotein subunit of the $(Na^+ + K^+)$-ATPase is closely associated with the ouabain-binding site of the enzyme. Transient kinetic studies have provided new and more direct evidence that the conformation of the transport ATPase is dependent upon the occupation of Na^+- and K^+-binding sites in addition to nucleotide binding and phosphorylation. An investigation of ouabain-sensitive cation fluxes in erythrocytes from subjects with myotonic dystrophy has found normal ratios of Na^+ flux to K^+ flux in contrast to a recently published report. Studies have been initiated in an effort to characterize endogenous tissue constituents that interact with the ouabain-receptor of the $(Na^+ + K^+)$-ATPase.

Project Description

Objectives: To assess the functions, mechanisms, and molecular structures of enzymes and enzyme systems that characterize neurons and other excitable cells and their supporting tissues. Current studies are directed toward extension of our knowledge of the mechanism and structure of the active transport system that establishes the electrochemical gradients of Na^+ and K^+ in excitable tissues. The immunochemical and transient kinetic studies have been extended during the current year.

Methods: Isolated plasma membrane fragments from brain and Electrophorus electric organ are the starting materials for our ($Na^+ + K^+$)-ATPase studies.

The further purification of the enzyme depends upon dissociation of the membranes with detergent. The detergent-solubilized enzyme can be isolated from other membrane proteins by preparative gel electrophoresis. The subunits are dissociated by sodium dodecyl sulfate and isolated by preparative gel electrophoresis.

Standard techniques for producing anti-sera in rabbits are employed using these proteins as antigens.

The rapid kinetics of phosphorylation and hydrolysis employ an apparatus designed by Dr. Froehlich of NIA which permits quenching of the enzymic reaction with acid with a time resolution of 3 milliseconds.

Steady-state kinetics employing spectrophotometric measurement of initial enzyme rates have been applied to a study of the pH and temperature effects on the conformation of the enzyme.

Radioactive ligands have been employed in a rapid filtration technique to explore the characteristics of cation interactions with the opiate receptor of brain membranes and a similar technique has been applied to a search for endogenous tissue constituents that interact with the ouabain-binding receptor of the ($Na^+ + K^+$)-ATPase.

Major Findings: (a) The specific antisera to the large and small subunits of the ($Na^+ + K^+$)-ATPase have been shown to act respectively as inhibitors of the phosphorylation of the large subunit and of ouabain binding to the enzyme. This reinforces our previous suggestions that the antigenic sites for these two antisera are on opposite sides of the plasma membrane and also establishes a close association between the glycoprotein subunit and the ouabain site.

(b) Transient kinetic studies have been extended to an examination of the conformational transitions that are induced by successive exposure of the enzyme to different ligands. This technique has demonstrated a relatively slow transition between the K^+-liganded state and the Na^+-

liganded states. We have also demonstrated a synergistic effect between Na^+ and ATP in this regard.

(c) We have initiated an investigation of those endogenous tissue constituents that appear to interact with the ouabain-binding site. We have made technical improvements that facilitate the assay. Most tissue extracts contain substances that retard ouabain binding. Characterization and evaluation of these materials is in progress.

(d) Mono- and divalent cations are known to interact with the opiate receptor of brain. We have initiated studies of the possible relation of these cation interactions to specific cation transport. We have found that the low level of receptor sites relative to non-specific binding of radioactive opiate ligands is a major obstacle in defining the characteristics of this receptor. We have been able to develop a procedure that substantially increases the precision of the binding assay. This is permitting more refined studies of the interaction of cations with this receptor.

Significance: Active Na^+ transport appears to be the principal metabolic activity of nerve cells. In most cases it is coupled to the accumulation of K^+. Extrusion of intracellular Na^+ is the basis for maintaining the resting membrane potential of nearly all animal cells. In the nervous system, it provides the linkage through which oxidative metabolism supplies energy to produce the nerve action potential, to release neurotransmitters, and to reaccumulate these transmitters or their metabolites.

With respect to the more general aspects of cell metabolism, Na^+ transport is closely linked to cellular uptake of carbohydrates, amino acids and other nutrients.

Various investigators have proposed that malfunctions of this system may be involved in such pathologics as epilepsy, myotonias, muscle atrophy, slow virus encephalopathy, etc.

Proposed Course: The transient kinetic studies will be continued in an effort to resolve the problems of the number and kinetic behavior of the individual steps of the reaction cycle.

The nature of the endogenous ligands that interact with ouabain-binding site of the ATPase will be pursued.

We expect to extend the characterization of the cation interactions with the opiate receptor. Experiments are planned to investigate the possible role of opiates in regulating Ca^{++} influx and efflux from neurons.

Publications:

1. Jean, D. H. and Albers, R. W.: Immunochemical studies on the large polypeptide of *Electrophorus* Electroplax (Na$^+$ + K$^+$)-ATPase. Biochim. *Biophys. Acta*. 452: 219-226, 1976.

2. Jean, D. H. and Albers, R. W.: Molecular organization of subunits of *Electroplax* (Na$^+$ + K$^+$)-activated ATPase. *J. Biol. Chem*. 252: 2450, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01586-10 LNC

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Trophic Neuronal Function in the Peripheral Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Zalewski	Act. Sec. Head, Neural Development and Regeneration	LNC	NINCDS

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LAB/BRANCH
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SECTION
Neural Development and Regeneration

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TOTAL MANYEARS: 2.2	PROFESSIONAL: 1.1	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the nature, and specificity or plasticity of trophic nerve function in the peripheral nervous system. Presently, the immunologically deficient nude mouse is being used as a recipient of xenografts (i.e., a graft from another species of animal) in order to study cross-species trophic nerve function. The results show that rat neurons can survive in xenografts in the nude mouse and that rat neurons can regenerate nerve fibers into mouse tongue tissue and induce the formation of taste buds. Other results demonstrate that rat muscle xenografts can survive and become reinnervated by motor nerve fibers of the nude mouse. It should be possible therefore to use the nude mouse as an "in vivo" recipient of human tissue grafts in order to determine whether the etiology of certain human neurological diseases (e.g., familial dysautonomia, congenital muscular dystrophy) involves an alteration of trophic function in nerve or end-organ.

Project Description:

Objectives: Trophic nerve function refers to the ability of a neuron to induce and maintain the development of an end-organ. The purpose of this project is to determine the nature (i.e., the neurochemical factors and end-organ receptors) and specificity or plasticity of normal or altered trophic function of neurons in the peripheral nervous system. In the present experiments we have begun to study cross-species trophic nerve function (i.e., the ability of neurons from one species of animal to reinnervate tissues of another species of animal). These cross-species studies were the outcome of previous work in which it was shown that (1) taste buds could regenerate in tongue tissue when tongue and ganglia grafts were cotransplanted to the anterior chamber of the eye (Zalewski, Exp. Neurol., 1972, 35: 519-528) whereas they could not adequately be grown in tissue culture (Oh and Zalewski, unpublished data), and (2) neurons in allografts (i.e., a graft from a genetically different animal of the same species) could induce taste buds in tongue grafts if immune rejection of the allografted neurons was prevented (Zalewski and Silvers, Exp. Neurol., 1973, 41: 777-781). It was while searching for methods of allograft immunosuppression that the discovery of the existence of the immunologically deficient nude mouse was made and its potential to serve as a recipient for a cross-species graft realized. The nude mouse is immunologically deficient because it is congenitally athymic (Pantelouris, Immunol., 1971, 20: 247-252) with the consequence that its T-lymphocytes do not mature and acquire the capability to reject a foreign graft regardless of whether it is an allograft, a xenograft (i.e., a graft from another species of animal) or a combination of both. Experiments were therefore begun to determine if cross-species taste bud induction and cross-species neuromuscular reinnervation could occur. The value of these studies is that they would indicate whether similar cell recognition molecules (i.e., trophic substances and receptors) were involved in neuronal trophic function regarding taste buds and muscle. More importantly the success of obtaining in vivo cross-species trophic nerve function would pave the way for testing tissues from human diseases in which trophic function may be absent or altered. For example, taste buds are absent in the disease familial dysautonomia (Pearson et al., Ann. N. Y. Acad. Sci., 1974, 228: 288-300) and it is not known whether the neurons or the tongue tissue is defective. Also various congenital neuromuscular diseases (e.g., muscular dystrophy) could be examined to elucidate whether the disease is neurogenic or myogenic in etiology. It would even be interesting to see if the autoimmune changes caused in muscle in myasthenia gravis could be reversed after grafting to the nude mouse since the immunologically deficient animal would not develop an immune response to the graft. Although Crain (Ann. N. Y. Acad. Sci., 1974, 228: 6-34) has obtained evidence of cross-species neuromuscular reinnervation in culture between rodent and human tissues it is not certain whether the muscle is mature. For example, it has not been shown yet that cultured cross-species reinnervated muscle differentiates into histochemical fiber types. Furthermore, there may be in vivo factors which influences the expression of a disease which could not be reproduced in culture but which would be present in the nude mouse (e.g., the degree of work a muscle does, hormones, other circulating factors, etc.)

Methods Employed: Nude mice, 6 weeks old, were obtained from the Small Animal Section maintained at the NIH. In some animals a xenograft of rat nodose ganglion and an allograft of tongue tissues from a normal C57BL-6 mouse that contained the taste bud bearing vallate papilla were cotransplanted to the anterior chamber of the eyes of nude mice by techniques previously used (Zalewski, Exp. Neurol., 1972, 35: 519-528). Other nude mice received a mouse tongue allograft only. Rat ganglia were also put into the eyes of immunologically normal C57BL-6 mice to determine if rat ganglia xenografts truly are antigenic. After various times frozen sections of the ganglia were examined for the presence of neurons and an immune response and tongue grafts were studied for the presence or absence of taste buds. The study of cross-species neuromuscular reinnervation was performed by transplanting the intact extensor digitorum longus (EDL) muscle into the site formerly occupied by the excised EDL muscle of the nude mouse. The EDL muscle of a 20 day old Osborne-Mendel rat was chosen as the xenograft because the muscle is mature and fits properly into the transplantation site. The muscle transplantation technique of Carlson and Gutmann (Anat. Rec., 1975, 183: 47-62) was used. In this study it was hoped that the motor nerve fibers of the nude mouse already present at the graft site would reinnervate the muscle xenograft. The effect of denervation on the rat muscle was examined by denervating the graft site by cutting the common peroneal nerve. Rat xenografts were also studied in nude mice that were immunologically reconstituted by giving them a thymus gland graft. Finally rat EDL muscle was grafted into immunologically normal C57BL-6 mice to illustrate the antigenicity of rat muscle. Frozen sections of the grafts were subsequently examined for the presence of muscle fibers and an immune response, for differentiation of histochemical muscle fiber types, and for neuromuscular junctions.

Major Findings: The rat ganglia transplanted to normal mice were rejected in that no neurons were present and the ganglia were infiltrated by immune cells. Taste buds were absent in the mouse tongue grafts transplanted alone (i.e., without added rat ganglia) to the eyes of nude mice. Thus, the original taste buds in the tongue disappeared since they were separated from this nerve supply and in the absence of innervation no buds spontaneously reappeared. On the other hand, neurons not only survived in rat ganglia that were transplanted into the eyes of nude mouse but they regenerated nerve fibers into the cotransplanted mouse tongue grafts and induced the formation of taste buds. Furthermore, there was no immune reaction to either the mouse tongue allografts or the rat ganglia xenografts. Rat muscle xenografts also survived and became reinnervated by the motor nerve fibers of the nude mouse after grafting to this animal. Many muscle fibers were present although they varied somewhat in size. Occasionally, foci of infiltrating cells were present, but if this represented an immune response by immature immune cells it was inadequate to cause xenograft rejection.

Neuromuscular junctions were readily found in these xenografts thereby demonstrating their innervation. Mature and differentiated muscle fiber types were present as identified after myosin ATPase and succinic dehydrogenase staining. Rat muscle xenografts also survived in the denervated leg of the nude mouse but these grafts were markedly atrophic, had no neuromuscular junctions, and no muscle fiber types were differentiated. The rat xenografts were rejected, however, in the immunologically reconstructed thymus gland-grafted nude mice just like they were rejected in normal C57BL-6 mice. The rejected muscles in both instances lacked muscle fibers and were heavily infiltrated by immune cells.

Significance:

1. The presence of neurons in rat ganglia and muscle fibers in rat muscle xenografts means that xenografted cells can survive in the nude mouse. The longest time to date the rat neurons have been studied was 60 days while rat muscle grafts have survived at 112 days. Mouse tongue allografts were examined at 35-40 days, the time we have found maximally required to form regenerated buds. Thus, xenografts can survive for prolonged periods in the immunologically nude mouse.

2. The induction of taste buds by xenografted neurons means similar molecular recognition molecules and mechanisms must be operative in the trophic function of sensory neurons on taste bud induction.

3. The survival of rat muscle fibers and their reinnervation by motor nerve fibers of the nude mouse again means a similar recognition mechanism is operative in motor neuron trophic function regarding muscle.

4. Other studies have also shown that monkey neurons and monkey taste buds can survive and regenerate respectively in xenografts. The survival of tissues from a diverse group of genetically different animals (i.e., mouse, rat, monkey) in the nude mouse should mean human tissue grafts can be expected to survive after in vivo transplantation to the nude mouse. The nude mouse should, therefore, be a valuable model to complement tissue culture as a means of studying possible alterations of trophic nerve function in human sensory, neuromuscular, or in autoimmune diseases.

Proposed Course of Project:

A) Taste Buds

   1) Obtain human tongue tissue to determine if taste buds can be induced in it when transplanted into the eyes of nude mouse (tissue can be obtained from Clinical Pathology at NIH). Initial histological observations have been made on normal human

tongue tissue. Later, dysautonomic tongue tissue can be tested for a trophic defect.

2) Determine the histochemical profile of cross species induced buds. This study will elucidate whether the innervating neurons merely induce buds or, in addition, convert buds to other histochemical types.

3) Perform electron microscopical studies on cross-species buds.

4) Consideration will be given to seeking collaboration in performing physiological studies on cross-species buds. In this instance it would be desirable to reinnervate the tongue in situ. This reinnervation can be done by implanting a xenograft of ganglion into a neck muscle of the nude mouse (initial studies have shown neurons can survive in rat ganglia implanted into the sternomastoid muscle) and joining it to the distal peripheral nerve stump of the glossopharyngeal nerve which innervates the taste bud bearing vallate papilla of the nude mouse (Zalewski, Transplantation, 1972, 14: 618-623.

5) Other studies concerning taste buds:

   a) Continue to try to grow taste buds in tissue culture (to be done in collaboration with Dr. Oh, University of Maryland).

   b) Perform grafting experiments in which tongue grafts from the anterior part of the tongue are combined with neurons which normally innervate posterior tongue tissue. The purpose of this study is to see if posterior neurons are merely inductive or whether they will, in addition, change the histochemical profile of anterior tongue taste buds so that the buds appear like posterior ones.

   c) Test the specificity of various epithelia and connective tissue in participating with the neuron in forming taste buds. For example, tongue and skin tissue will be trypsinized to separate epithelium and dermis and various recombinations made. Could the nerve now induce taste buds in skin epithelium growing on tongue dermis or are epithelium and dermis of tongue both required to form buds?

   d) Determine if various lectins bind to receptors involved in taste bud formation which might provide insight into the nature of trophic receptors.

B) Muscle:

1) Obtain normal human muscle to see if it can survive and become reinnervated after transplantation to the nude mouse. If it does, the defects of various neuromuscular disorders (Bender and Bender, Neurol., 1977, 27: 206-212) might be reversed upon reinnervation by normal nerve fibers thereby elucidating whether a disease is neurogenic or myogenic.

2) Determine whether muscle grafts can synthesize new acetylcholine receptors (AChR). If it can, muscle from patients with myasthenia gravis can be tested to determine why this muscle has a reduced number of AChR at the end-plate. It is uncertain at present whether a normal number of AChR are present and some are blocked or destroyed by the autoimmune process present in this disease.

C) Nerve:

1) Determine whether there is any specificity of neurilemmal cells (i.e., Schwann cells) in allowing the nerve fibers they surround to perform neuronal trophic function. For example, could motor nerve fibers of the nude mouse regenerate through a rat or avian (quail) nerve graft and become myelinated and then reinnervate the muscle?

Publications:

1. Zalewski, A. A.: The neural induction of taste buds in the salivary ducts of the lingual gland of Von Ebner. Exp. Neurol. 52: 565-580, 1976.

2. Zalewski, A. A., Creswell, G. F., Goshgarian, H. G., and Oh, T. H.: The nude mouse: An *in vivo* model for demonstrating cross-species trophic nerve function. Exp. Neurol. 54: 397-402, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01885-07 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Kinetics of Brain Glycogen

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The changes in glycogen concentrations during and following ischemic episodes were studied. Mongolian gerbils were made ischemic by occlusion of the left carotid artery. After either 1 or 3 hours of occlusion, glycogen levels in the ischemic cortex were 10% of control. Following recirculation, glycogen levels were normal after 1 hour in both ischemic groups. After 1 week of recirculation, glycogen levels were twice normal concentrations in animals that had been ischemic 1 hour. In the 3 hour ischemic group, glycogen concentrations increased to 170% of control after 5 hours of recirculation. In this group, glycogen was still elevated after 20 hours or 1 week of recirculation.

This project has been completed.

Project Description:

Objectives: To further explore factors in the turnover, accumulation and disappearance of glycogen in the brain. To determine the effects of trauma, drugs, and altered physiological states such as hypothermia, ischemia, or drug administration on glycogen metabolism.

Methods Employed: Mongolian gerbils, 50-60 g were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and unilateral ischemia was produced by occluding the left common carotid artery with an aneurysm clip. The animals with neurological symptoms were frozen after 1 or 3 hours of ischemia, or after 1 hour to 1 week of recirculation. The cerebral cortex was excised at -20° from the hemispheres ipsilateral or contralateral to the occluded artery. Sham-operated animals were also used as controls. The tissue was extracted in 0.03N HCl and the glycogen measured by an enzymic procedure.

Major Findings: The glycogen concentration in the contralateral cerebral cortex was essentially the same as that of sham-operated gerbils (19 nmoles/mg protein). Occlusion of the common carotid reduced the glycogen concentration to 1.5 or 1.8 nmoles/mg protein after 1 or 3 hours of ischemia. After 1 hour of recirculation, glycogen concentration appeared normal in both groups of animals previously made ischemic. In the 1 hour ischemic group glycogen appeared normal until after 1 week of recirculation. At this time, the glycogen was twice the normal concentration. After 3 hours of ischemia, and 5 hours of recirculation, cerebral glycogen was increased 170% over control values. After 20 hours and 1 week of circulation, the glycogen stores in this group were still elevated.

Significance to Biomedical Research and the Program of the Institute: The decreases in glycogen stores were comparable after 1 or 3 hours of ischemia. Following recirculation the restoration to normal values required 1 hour. However, in the 3 hour ischemic group, glycogen concentrations increased above normal after 5 hours of recirculation and persisted for 1 week. In the 1 hour ischemic group, the glycogen appeared normal until after 1 week of recirculation. The biochemical events following recirculation appear to reflect the severity of ischemic insult more accurately than those changes occurring during the ischemic period. Once the ischemic threshold is reached, the length of time required for the development of lesions is an inverse function of the length of the initial ischemic interval. Lesions appear earlier after longer periods of ischemia.

Proposed Course of the Project: This project has been completed.

Publications: Mrsulja, B. B., Lust, W. D., Mrsulja, B. J., Passonneau, J. V., and Klatzo, I.: Brain glycogen following experimental cerebral ischemia; biochemical evidence for maturation phenomenon. Experientia. 36: 732, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01942-06 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of Cyclic AMP and Cyclic GMP in the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. D. Lust	Research Pharmacologist	LNC	NINCDS
OTHER:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDS
	A. Wheaton	Biological Lab Technician (Micro)	LNC	NINCDS

COOPERATING UNITS (if any)

None

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Laboratory of Neurochemistry

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The levels of cyclic nucleotides were determined in both cerebellum and cerebral cortex following either pharmacological treatment or during electrically-induced seizures. Generally, neurotropic agents which either depress the CNS or protect against seizures decrease cerebellum cyclic GMP and agents which stimulate the CNS increase the cyclic GMP. Cyclic AMP is not pharmacologicall responsive in either region. Electroconvulsive shock increases the levels of both cyclic nucleotides in both the cerebellum and the cerebral cortex. However, there is a temporal disparity in the cyclic nucleotide responses which favors the concept that the two cyclic nucleotides have separate and distinct roles in the CNS. Halothane, an inhalation anesthetic, increased the levels of cyclic GMP in the cerebral cortex and decreased them in the cerebellum. This effect is similar to that observed with atropine which may indicate a cholinergic component to the action of Halothane.

Project Description:

Objectives: To determine if either pharmacological or physiological alterations of brain metabolism would affect the steady state levels of cyclic AMP and/or cyclic GMP *in vivo*.

Methods Employed: Mice were rapidly frozen in liquid nitrogen at the appropriate times following treatment. The brains were removed at -25°, weighed and extracted in perchloric acid. The neutralized PCA extracts were used in all subsequent metabolic measurements. Cyclic GMP was measured by an enzymic-cycling technique or by the immunoassay method of Steiner, and cyclic AMP was measured by a radioactive binding assay.

Electroconvulsive shock (ECS) was applied by corneal electrodes at an intensity of 50 mA for a duration of 0.2 sec. The electroshock produces a convulsive response manifested by (a) a tonic extensor phase (0-15 sec.), (b) an intermittent clonic phase (15-30 sec.), and (c) a postictal depressive phase (> 30 sec.).

In the thermal experiments, the mice were rendered (a) hypothermic (20°) by forcing them to swim for 1-2 minutes in a water back followed by a 10 minute stay in a cold chamber, and (b) hyperthermic (41°) by placing them under a heat lamp. Rectal temperatures were monitored with a telethermometer.

Major Findings: The continuation of the work on electroconvulsive shock (ECS) and ischemia (produced by decapitation) confirmed our previous findings that the levels of cyclic AMP increase rapidly following both treatments in all areas of the brain tested. The levels of cyclic GMP in the cerebellum also increase almost 4-fold following ECS. The major difference between the cyclic nucleotide responses in the cerebellum is that the maximal change in cyclic AMP occurs at 15 seconds and precedes the cyclic GMP peak by approximately 45 seconds. Relating the cerebellar cyclic nucleotide responses to convulsive behavior, it is apparent that the major cyclic AMP changes occur during the excitable states (tonic, 0-15 sec. and clonic, 15-30 sec.), and cyclic GMP during the depressive state (postictal, greater than 30 sec.). Although the levels of cyclic nucleotides drop rapidly following their maximal ECS-induced changes, the restoration of cyclic AMP and cyclic GMP to control levels was not reached by 240 seconds. In addition, the cyclic AMP response to ECS differs in the cerebral cortex and the cerebellum. The cyclic AMP response in the cerebral cortex is 2-3 fold greater than that in the cerebellum and the peak response occurs at 45 sec. post-ECS or some 30 seconds after the cerebellar maximum. In contrast, the cyclic AMP increase following decapitation is greater in the cerebellum than in the cerebral cortex.

In pharmacological studies, phenobarbital, papaverine and $MgSO_4$ significantly decreased the levels of cerebellar cyclic GMP. Phenobarbital (100 mg/kg IP) lowered cyclic GMP within 5 minutes and the levels remained at 20% of control for up to 90 minutes. Of all the drugs tested, only chlorpromazine and trifluopromazine lowered cyclic AMP levels. Pretreatment with two drugs, amphetamine (10 mg/kg IP) which elevates GMP 2-fold, or phenobarbital (20 mg/kg IP) which decreases cyclic GMP 50%, had no effect on the ECS-induced increase in cyclic nucleotides, in spite of significant alterations in overt convulsive behavior.

The anti-convulsant agents, phenytoin, clonazepam (C) and dipropylacetic acid (DPA) decreased cerebellar cyclic GMP levels by more than 50%. Conversely, the convulsant agents, isoniazid (INH), pentylenetetrazol (PTZ) and theophylline increased the cyclic GMP levels by at least 3-fold. And in combination, the convulsant-induced elevation of cyclic GMP was inhibited by the anti-convulsant agents. Specifically, DPA and C blocked the INH-induced increase in cyclic GMP, and C blocked the PTZ-induced increase. Thus, these anti-convulsant agents were not only able to block the chemically-induced seizures, but also the increase in cerebellar cyclic GMP.

A time course following 400 mg/kg IP of DPA provides additional evidence for a relationship between anti-convulsant activity and cerebellar cyclic GMP levels. Anti-convulsant activity here was defined as the ability to abolish tonic extension following ECS. The levels of cyclic GMP were depressed at 1/2 hour and 1 hour after injection when more than 60% of the mice were protected against seizures. As anti-convulsant activity diminished at 2 and 4 hours, the cyclic GMP returned to control levels. So, the lowered cyclic GMP levels coincided temporally with the anti-convulsant activity.

Additional studies with anti-convulsants demonstrated that all the agents with the exception of Diamox depressed cerebellar cyclic GMP levels. Four anti-convulsants were found to have no effect on the levels of cerebral cortica cyclic GMP. In contrast, the convulsants, isoniazid and pentylenetetrazol, increased the levels of cyclic GMP in the cerebral cortex, as well as the cerebellum. ECS also increased the levels of cyclic GMP some 5-fold in the cerebral cortex.

Mice pretreated with 20 mg/kg of phenytoin exhibited a clonic-type convulsion instead of the tonic extension normally observed following ECS. In spite of the alterations in convulsive behavior, the ECS-induced changes in cyclic nucleotides were only minimally affected by the agent. The cyclic GMP elevation in the cerebellum was reduced by 50%, whereas the cyclic AMP changes and the cyclic GMP increase in the cerebral cortex exhibited little or no change.

The effect of a 15 minute exposure to the inhalation anesthetic, Halothane, on the levels of cyclic nucleotides in three regions of the nervous system was investigated. The levels of cerebellar cyclic GMP decreased to les than 10 percent of the control values at a halothane concentration of 1.5 percent or greater. In contrast, at the same doses of halothane the levels of cyclic GMP in the cerebral cortex increased almost 5-fold. In the spinal cord, the levels of cyclic GMP decreased slightly. The response of cyclic GMP in the cerebellum and cerebral cortex is quite similar to that reported following treatment with atropine. As observed with other neurotropic agents, cyclic AMP did not appear to be pharmacologically responsive.

The levels of cyclic nucleotides were also measured in three brain region of the wobbler mouse. The wobbler mouse has been considered an animal model of spontaneous lower motor neuron degeneration which is inherited as an autosomal recessive trait. Cyclic GMP levels were decreased to 71, 43 and 20 percent of control values in the cerebral cortex, cerebellum and spinal cord, respectively. The concentration of cyclic AMP was unaffected. Glucose and glycogen levels were elevated in the cerebellum but other metabolites includin GABA, P-creatine and ATP were not significantly different from control.

Significance to Biomedical Research and the Program of the Institute: Both the ECS and pharmacological data favor the argument that cyclic AMP and cyclic GMP are independently regulated in vivo. The possibility of a direct relationship between elevated cyclic GMP and depression seems remote, since pharmacologically-induced depression either reduced or had no effect on cyclic GMP levels. There is an increasing amount of evidence favoring an association between either lowered cyclic AMP or elevated cyclic GMP and CNS excitation; and between elevated cyclic AMP or lowered cyclic GMP and CNS depression. Thus, neural events following a number of neurotropic agents or such treatments as ECS or brain injury might be defined by the cyclic nucleotide profiles. From our studies with pharmacological agents, the levels of cerebellar cyclic GMP appear to reflect a behavioral spectrum from depression to convulsion; CNS depressants and anti-convulsant agents lower cyclic GMP while convulsant agents elevate cyclic GMP. Thus, cyclic GMP in the cerebellum may serve as a molecular indicator of the degree of CNS excitability, and therefore may be useful in our understanding of the mechanism of action of a variety of neurotropic drugs.

The ECS- and anoxia-induced changes in brain cyclic AMP exhibit regional variation. Thus, while anoxia produced a much greater accumulation of cyclic AMP in the cerebellum, electrical stimulation caused a much greater production of cyclic AMP in the cerebral cortex.

The cyclic nucleotide elevation following ECS has been attributed by others to be a result of the apnea-induced anoxia which occurs during tonic extension. The absence of a cyclic GMP increase in either cortex or cerebellum 15 seconds after decapitation would tend to refute this explanation. Further, reduction of the anoxic period during tonic extension by phenytoin had only a slight effect on the cyclic nucleotide response. These results would tend to decrease the likelihood that anoxia is the major stimulus to the cyclic nucleotide increases after electroshock.

Cyclic GMP in the cerebral cortex increases following electroshock or pretreatment with isoniazid and metrazol at convulsive doses. Why halothane at anesthetic doses produces an effect similar to that observed with convulsants is presently unclear. However, the response is similar to that for atropine and this effect may be useful in the explanation of the mechanism of action for halothane.

Cerebellar cyclic GMP in nervous mutants is approximately 20% of control values. The population of Purkinje cells in the cerebellum of these mice has been demonstrated to be quite low. The coincidental reduction of both cerebellar cyclic GMP and the number of Purkinje cells has been used as an argument for the cyclic GMP being located in the Purkinje cells. However, a similar reduction in cyclic GMP is demonstrable in the cerebellum from Wobbler mice and the lesion in these mice is thought to be in the spinal cord not the cerebellum. Thus, the localization of cyclic GMP in the Purkinje cells on the basis of depressed levels in nervous mutants is not very convincing.

Proposed Course of Project: Presently, the primary emphasis on the relationship of cerebellar cyclic GMP to the different types of anticonvulsants and their different anticonvulsant activities (anti-metrazol, anti-ECS, anti-bicuculline, etc.). Also, more extensive studies on the seizure-dependent rise of cyclic nucleotides, whether chemically- or electrically-induced are being undertaken in other regions and in distinct layers of those regions. Further studies on the long term effect of halothane anesthesia as opposed to the short-term study are in progress. Cyclic nucleotide levels are to measured in mice exposed to polio virus to determine the effect of a spinal cord lesion on these metabolites.

Publications: Nahrwold, M. L., Lust, W. D. and Passonneau, J. V.: Halothane-induced alterations of cyclic nucleotide concentrations in three regions of mouse nervous system. Anesthiology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02006-05 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation of Metabolism in Glioma and Neuroblastoma Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDS
OTHER:	W. D. Lust	Research Pharmacologist	LNC	NINCDS

COOPERATING UNITS (if any)

None

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Laboratory of Neurochemistry

SECTION
Section on Cellular Neurochemistry

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being carried out on 2 cell lines in culture. C-1300 neuroblastoma cells, and C-6 astrocytoma cells are used to investigate differences, if any between the metabolism of cells of glial and neural origin. The regulation of cyclic AMP and cyclic GMP concentrations in the cells, and the fluctuation of these compounds in response to hormones and/or drugs is under investigation. C-6 cells respond to norepinephrine, and C-1300 cells respond to prostaglandin E_1 in that adenyl cyclase is activated and cyclic AMP concentrations increase. A study of the effects of glucose deprivation and/or anaerobiasis has also been undertaken.

Project Description:

Objectives: To investigate the regulation of metabolism in a glioma and a neuroblastoma cell line.

Methods Employed: The cells are being grown in a CO_2 incubator in the laboratory. Homogenates of the cells are analyzed for glycolytic metabolites, as well as cyclic AMP, cyclic GMP, and pyridine nucleotides, and for enzymes such as glycogen synthetase and phosphorylase. All of these analytical methods have been used in the laboratory on whole brain and are thus easily adaptable for use with the cells.

Major Findings: The effects of hormones and drugs, such as depolarizing agents, on the metabolism of both cyclic AMP and cyclic GMP, have also been studied.

In addition to cyclic AMP metabolism, a study of the energy metabolism of both cell lines has been carried out. The kinetics of glucose uptake, of glycogen breakdown, and of pyruvate and lactate efflux and uptake have been studied. In addition, the effects of the local anesthetic, tetracaine, on this system have been studied. The cells are quite resistant to anaerobiasis and maintain ATP and P-creatine levels even under N_2 atmosphere, unless glucose is also excluded from the medium.

Significance to Biomedical Research and the Program of the Institute: A study of the regulation of metabolism in the brain is complicated by the presence of several cell types and the inability to determine in which cells metabolic alterations are occurring. Such studies are facilitated by the use of the two distinct cell lines, the glioma (rat C-6 line) and the neuroblastoma (mouse C-1300 line), because regulation can be studied in each cell line separately. Until these cell lines became available, for example, it had not been realized that glial cells might be hormone-responsive. Such results suggest that the glial cells may have a greater involvement in brain function than the supportive one originally envisaged. Although these two cell lines are tumor cells, they offer a first approach to the problem of regulation of metabolism in the brain, which might be followed up in normal brain cells when the isolation techniques for glial and neuronal cells have been perfected.

Proposed Course of the Project: A thorough investigation of the regulation of cyclic AMP and cyclic GMP levels, of other energy-related parameters, and of pyridine nucleotide ratios will be undertaken, in both cell lines. Studies on ion transport are also planned, as are morphological studies of the effects of various agents and growth conditions. The effects of glucose deprivation and/or anaerobiasis on both cell lines is being investigated. Further studies related to the effect of thiamine deficiency on thiamine-dependent enzymes and the pentose shunt are planned.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02077-04 LNC

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Physiological and Biochemical Correlates of Lipid Metabolism in Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. S. Goldman, Staff Fellow LNC NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH Laboratory of Neurochemistry

SECTION Section on Enzymes

INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this study to resolve the cold resistant nature of the brain of a mammalian hibernator. The topics of interest and investigation are: (1) Fatty acid metabolism, primarily that associated with the desaturation of fatty acids, (2) glycerophospholipid metabolism, (3) membrane lipid turnover, and (4) membrane biogenesis. The use of the hibernating species of mammal, the hamster, is employed in these studies since it can alter naturally when cold exposed, both the chemical and physical nature of its neural elements.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To elucidate the physiological and biochemical basis of membrane lipid turnover in brain.

Methods: The turnover of membrane lipids in brain is considered to be the result of a number of sequentially linked processes which results in the maintenance of a constancy of lipid in adult brain. (1) Synthesis, involving the de novo production of hydrocarbon chains followed by their assembly into a final glycerophosphatide. (2) Insertion of the lipid into the membrane replacing that which have been (3) catabolished as a result of synaptic activity and or normal replacement processes.

The strategy employed in these investigations is to compare a number of enzymatic systems associated with glycerophosphatide biosynthesis in warm-adapted hamsters with those of cold adapted (hibernating) hamsters. To this end a stock of hibernating hamsters is maintained.

Specific Methods: A working hypothesis of the current project is that alterations in membrane lipids are central to the cold adaptation phenomenon. It was shown that brain membranes prepared from hibernating hamsters possessed greater amounts of monounsaturated hydrocarbon (monenes) and were less viscid than brain microsomes prepared from warm-adapted hamsters. Since it is generally assumed that monenes are supplied to adult brain from either de novo synthesis and/or from peripheral sources the questions naturally follow: which of these to routes predominate in supplying the necessary monenes to meet the demand for the lipid adaptation in brain. Secondly, does a biochemical and/or physiological mechanism exist in which there is an increased selectivity process associated with monene esterification in glycerophosphatide synthesis.

Standard techniques involved with the isolation of both brain and liver membranes possessing acyl CoA desaturase and lysophosphatidic acid acyl CoA transferase activity were employed. Techniques involving standard as well as argentation silica gel thin-layer chromatography, difference spectroscopy, and gas-liquid chromatography were also employed.

Major Findings: In the past year the apparent source of monenes that accounts for the lipid adaptation in brain was described. The enzyme associated with monene production, acyl CoA desaturase, and its associated microsomal electron transport chain (cytochrome b_5 reductase and cytochrome b_5) was investigated in both liver and brain. The activity of the brain enzyme and microsomal electron transport chain was no different in either the warm adapted or hibernating hamster. The activity of the enzyme from liver was 8 X greater in hibernating hamster without concomittant increases in the microsomal electron transport chain. Further evidence indicates that the serum levels of free monenes was also greater in both hibernating and aroused hibernating hamsters when compared to warm-adapted hamsters. It was concluded that de novo monene production in brain alone cannot fully account for the supply of monenes to support the lipid adaptation, and that liver must be the primary source of monenes.

Further studies were involved with the lysophosphatidic acid acyl CoA transferase and phospholipase A-2 (deacylation-reacylation) cycle. Preliminary findings indicate that the rate of phosphatidic acid formation and the esterification of monounsaturated fatty acid to the C-2 position on lysophosphatidic acid are both more intense in brain microsomes prepared from the hibernating hamster. The activity of the reacylation reaction associated with the phospholipase A-2 cycle also appears to favor monounsaturated fatty acids to a greater extent in membranes prepared from the hibernating hamster.

Significance: The significance of the past year's work is useful in regard to understanding the flow of hydrocarbon moieties into brain membrane lipid. It is apparent from these studies as well as those reported from other laboratories that de novo synthesis of both saturated and monounsaturated fatty acid production in adult brain only in part accounts for the fatty acids needed for replacement as a result of membrane turnover. Peripheral sources of fatty acid are also important and this is especially true for monounsaturated fatty acids in hibernators.

It is apparent from these studies that the primary source of monounsaturated fatty acid is the liver. Of secondary significance are the number of probable sites associated with the increased selectivity of monene esterification during glycerophosphatide synthesis in brain. Firstly, and an unknown entity itself, is whether the "blood-brain barrier" can discriminate between saturated and unsaturated fatty acid and, if so, is there greater discrimination in hibernating species of mammals. Secondly, in in vivo a biochemical mechanism exists whereby the C-1 position of glycerol-3-phosphate is predominately esterified with a saturated fatty acid and the C-2 position with an unsaturated fatty acid. In in vitro this is not the case for adult brain, whereas the hibernator possesses a more vigorous incorporation of monounsaturated fatty acid into the C-2 position. The same also appears to be true for the phospholipase A2 cycle. Thus the use of the hibernator as a tool may be helpful in resolving many of the unanswered basic issues that today exist with regard to phospholipid metabolism in adult brain.

Since the lipid adaptation apparently involves both myelin sheath and neural membrane lipids, it is conceivable that the adaptation encompasses a restructuring process of both nerve cell membrane and myelin sheath lipid. An understanding of these events would be of paramount importance in elucidating those events associated with the turnover of neuronal membrane in general.

Proposed Course: This project will be terminated 30 Sep 1977.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02141-03 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Glycogen Metabolism in Glioma and Neuroblastoma Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. V. Passonneau   Head, Sect. on Cell. Neurochem.   LNC   NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemisty

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS   ☐ (b) HUMAN TISSUES   ☒ (c) NEITHER

☐ (a1) MINORS   ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regulation of glycogen metabolism has been studied in C-6 astrocytoma and C-1300 neuroblastoma cell lines in culture. There appear to be two principal modes of controlling glycogen synthesis and breakdown. The concentrations of glycogen in the cells, and the amount of glycogen phosphorlase and glycogen synthase in the active forms can be regulated by the available glucose supply; however, there is a maximum level of glycogen which is not exceeded even in the presence of greatly increased glucose supply. When glucose in the medium falls below a critical level, phosphorylase is activated and glycogenolysis occurs. Substances which cause cyclic 3', 5'-AMP to be elevated in the cells also stimulate glycogenolysis. In C-6 glioma cells, norepinephrine is effective; in C-1300 neuroblastoma cells, prostaglandin E, is effective. Both cell lines respond to the phosphodiesterase inhibitor, isobutyl methylxanthine, and the adenyl cyclase stimulator adenosine.

This project has been completed.

Project Description:

Objectives: To determine the factors regulating glycogen metabolism in glioma and neuroblastoma cell lines.

Methods Employed: Two cloned cell lines, C-6 astrocytoma cells (rat brain) and C-1300 neuroblastoma cells (mouse cord) are grown in a CO_2 incubator. Cells are rapidly fixed by freezing in liquid nitrogen to preserve the *in vivo* state. Homogenates of the cells are analyzed for total glycogen phosphorylase and total glycogen synthase, as well as the per cent of those enzymes which are in the a or active form. Extracts of cells grown under identical conditions are analyzed for glycogen, glucose, glucose 6-P, uridine diphosphoglucose and cyclic 3', 5'-AMP (cyclic AMP) content. The two cell lines have been investigated with regard to response to available energy sources in the medium, as well as the influence of phosphodiesterase inhibitors, stimulators of adenyl cyclase, and insulin.

Major Findings: The concentration of glycogen in C-6 glioma cells and C-1300 neuroblastoma cells is regulated in part by available energy sources, in this case glucose. However, there is a maximum level of glycogen which is not exceeded, even if the glucose concentration is increased from 5 to 50 mM in the medium. The rate of formation and concentration of glycogen in the cells are the same in either case. The peak level of glycogen in C-6 glioma cells is twice that of the neuroblastoma cells; the concentrations are 100-120 and 40-50 nmoles/mg protein in the C-6 and C-1300 cells, respectively. Similarly, the amount of glycogen synthase in the C-6 cells is twice that of the C-1300 cells, and the glycogen is accumulated faster in the C-6 cells. If insulin is added in the presence of glucose, intracellular concentrations of glucose increase; the amount of glycogen phosphorylase, the degradative enzyme, in the active form is decreased; the amount of glycogen synthase (the synthetic enzyme) in the active form is increased; and glycogen stores increase. All of these changes, which are related to available nutrients, occur without alterations in cyclic AMP concentrations in the cells.

However, the cells will respond to a variety of stimuli; certain hormones, phosphodiesterase inhibitors, or stimulators of adenyl cyclase, cause intracellular concentrations of cyclic AMP to increase. In the case of norepinephrine, cyclic AMP increases more than 10-fold in C-6 cells and phosphorylase is converted primarily to the active form (4-fold increase), synthase to the inactive form and glycogenolysis occurs. Norepinephrine had no effect on C-1300 neuroblastoma cells. Prostaglandin E_1 was effective in raising cyclic AMP concentrations in C-1300 neuroblastoma cells. Isobutyl methylxanthine, a phosphodiesterase inhibitor, increased cyclic AMP in both cell lines, with consequent phosphorylase activation and degradation of glycogen. Adenosine, an activator of adenyl cyclase, potentiated the effect of isobutyl methylxanthine in C-6 astrocytoma cells.

Significance to Biomedical Research and the Program of the Institute: It has been demonstrated in these two cell lines of neural origin that regulation of glycogen metabolism can be exerted by regulation of supply of glucose. Previously, in mammalian systems, we showed that brain glycogen would increase if blood glucose levels were maintained at high concentrations, and if insulin were present. No glycogen increases in the brain could be demonstrated in alloxan diabetic mice, although blood glucose levels were 10-fold greater than normal. The demonstration of insulin effects in neural tissue is of great importance and has not previously been shown without ambiguity. In addition, the response of the cells to hormones and drugs has shown that the cyclic AMP activating system for phosphorylase can be controlled by a variety of agents. The response of the glycogen synthesizing and degrading system is thus under two modes of control which permits glycogen to be stored under ordinary conditions, and mobilized in response to stimulatory agents.

Proposed Course of the Project: This project has been completed.

Publications: Passonneau, J. V.: Glucose transport and metabolism in cultured cells of nervous tissue. *J. Cell. Physiol.* 89: 693-696, 1976.

Project Description:

Objectives: To determine the alteration in enzymes, metabolites and putative neurotransmitters during unilateral and bilateral ischemia in the gerbil cerebral cortex; and further, to monitor the recovery of these metabolites following a period of ischemia. To relate the changes in the biochemical parameters to the impairment of function which occurs following ischemia. To investigate the effects of ischemic cerebral hemispheres on non-ischemic regions of the nervous system.

Methods Employed: Mongolian gerbils were anesthetized, the common carotid arteries were exposed and looped with suture thread. As the gerbils emerged from the anesthesia, the artery(ies) was ligated. Those animals which exhibited positive neurological signs were frozen at various times following ligation in liquid nitrogen.

The cerebral cortex was removed at -20° and extracted in perchloric acid. In the unilateral ischemia studies, the left hemisphere served as the ischemic side and the right as the control. ATP, P-creatine, glucose, glycogen, glutamate, citrate and GABA were determined enzymically. Cyclic nucleotides were measured fluorometrically.

In the recovery studies, the carotid artery was occluded with an aneurysm clip for the appropriate time and then released. At various times following release, the gerbils were frozen and the brains removed as described above.

Major Findings: The investigation of ischemia is split into two groups: 1) unilateral ligation for long-term ischemia studies and 2) bilateral ligation for short-term studies. Although the duration of unilateral ischemia usually exceeded those periods currently thought to be consistent with survival, both approaches contributed to our understanding of the biochemical events which occur during ischemia.

Unilateral ischemia. By most of the criteria previously established in the decapitated mouse brains, the left cerebral cortex following the ligation of the left common carotid artery is, in fact, ischemic. From 1/2 to 6 hours after ligation, the ATP, P-creatine, glycogen and glucose were decreased by 60% or more and remained low for the entire period. The right or control cerebral cortex was essentially the same as a sham-operated control for up to 6 hours.

Cyclic AMP in the ischemic side increased 7-fold to a maximum at 2 hours and thereafter decreased to control values by 6 hours. GABA increased with time in the ischemic cerebral cortex to a maximum 5-fold greater than control at 6 hours. In addition, dopamine, norepinephrine and serotonin decreased with increasing periods of ligation. The decrease in dopamine and norepinephrine was significant within 30 minutes, while that for serotonin was only significant after 3 hours of ischemia.

In the recovery studies, the metabolites were measured at 5 minutes, 1, 5 and 20 hours after either 1 or 3 hours of ischemia. All the metabolites measured were essentially back to control levels by 1 hour of recovery. Cyclic AMP increased an additional 5-fold over the already elevated cyclic AMP levels after 5 min of recovery; but was restored to normal values by 1 hour of recirculation.

Bilateral ischemia. The results for bilateral ischema of 1, 5 and 20 minutes were qualitatively similar to those observed with unilateral ischemia. However, since the first sampling in unilateral ischemia was at 30 minutes after ligation, the biochemical events which occurred in the earlier stages of ischemia were obscured. The levels of ATP, P-creatine, glycogen and glucose were already maximally depressed after 1 minute of ischemia. Cyclic AMP levels increased 19-fold in the first minute of ischemia and thereafter decreased to a concentration approximately 5-fold greater than control values at 5 minutes of ischemia. Thus, the time course of the cyclic AMP changes during ischemia is marked by an early large rise, followed by a drop of cyclic AMP to a plateau some 5-fold greater than control which persists for up to 2 hours and finally by a gradual decrease to control levels at 6 hours of ischemia.

Upon recirculation, restoration of P-creatine and glucose after both 1 and 5 minutes of recovery was essentially complete in all groups. In the 5 and 20 minute ischemic group, there was a marked overshoot of the metabolites during recovery. Although the levels of ATP in the 1 and 5 minute groups approached control values during recirculation, restoration was never complete. In the 20 minute ischemic group, the recovery of ATP was severely compromised, but the overshoot of glucose and P-creatine was still evident after 30 minutes of recirculation. All of the gerbils in this group died by 60 minutes of recirculation.

Regionals effects. The levels of the various brain metabolites were measured in the spinal cord and cerebellum in gerbils with ischemic hemispheres. The maintenance of ATP and P-creatine levels suggest that these regions were not rendered ischemic by the bilateral ligation of the common carotid arteries. Both lactate and glucose were elevated during and after ligation in the cerebellum but not the spinal cord. In the 20 minute ischemic group, cerebellar cyclic GMP was increased, a condition similar to that observed in convulsing mice. Cyclic AMP concentrations in the spinal cord were depressed in all groups. Thus, the ischemic cerebral hemispheres have an effect on the metabolites from a non-ischemic region. Whether these alterations are mediated by neuronal pathways is presently unclear.

Repeated ischemia. Gerbils received a one hour unilateral ischemic insult. If the animals exhibited positive neurological signs, the clips were removed for 1, 5 or 20 hours after which the same carotid artery was reoccluded. Based on the metabolite measurements from these animals, the responses to the second ischemic episode fit into two distinct categories; 1) hyperresponsive after 1 hour of recirculation and 2) hyporesponsive after 5 and 20 hours of recirculation.

5% oxygen atmosphere: In preliminary studies, the cerebral metabolites in animals exposed to 5 minutes of 5% oxygen without other treatment were unaffected. Another group of gerbils were submitted to unilateral ligation followed by a period of low oxygen tension (5%). In those animals which did not exhibit neurological signs a subsequent five minute exposure to a 5% oxygen atmosphere induced neurological signs in 67% of the animals. Based on the presence or absence of neurological signs, as well as the time of onset, there appear to be distinct metabolite responses which may be a function of the anoxic or hypoxic insult.

Significance to Biomedical Research and the Program of the Institute: The gerbil model for the investigation of ischemia is quite useful for evaluating the role of energy metabolism and cyclic nucleotide in irreversible brain damage. Because a substantial fraction of the gerbils have an anomaly of the arterial circulation in the brain, these animals can be rendered ischemic by carotid ligation. The size of the gerbil permits relatively rapid fixation of the brain in liquid nitrogen for the investigation of brain metabolites. In both unilateral and bilateral ischemia, there is a large derangement of energy metabolites, cyclic nucleotides and certain putative neurotransmitters. Only in the case of the neurotransmitters is the change in concentration proportional to the ischemic duration. After the initial rapid changes in energy metabolites and cyclic nucleotides, the concentrations remain essentially constant. Perhaps the best indicator of the extent of ischemia is the rate of recovery of the energy metabolites. In fact, the restoration process may be an important factor to understanding critical events which lead to the loss of function.

The large changes in cyclic nucleotides probably reflect the severe biochemical perturbations which occur in the brain both during and after ischemia. It is quite evident that certain events must occur in the ischemic cerebral cortex between 1 and 5 minutes of ischemia which permit the large post-ischemic accumulation of cyclic AMP observed at 5 minutes of recirculation after 5 but not 1 minute of ischemia. The current concept is that certain adenylate cyclase agonists are being liberated during the first five minutes of ischemia, but that their expression is not manifested (in terms of cyclic AMP synthesis) because of the low levels of the substrate for adenylate cyclase, ATP. Upon recirculation, the ATP levels are rapidly regenerated and in the presence of the available agonists a burst of cyclic AMP production ensues. Thus, a proper temporal relationship between the loss of ATP and the liberation of adenylate cyclase agonists is critical to the cyclic AMP accumulation during recirculation. Cyclic GMP levels decrease during ischemia and remain low during recirculation except for 1 and 5 minutes of recirculation when there is a 4-fold increase. Based on electrophysiological results from other laboratories, it would appear that neuronal excitability is reduced by cyclic AMP and increased by cyclic GMP. Therefore, the elevated cyclic AMP levels and the reduced cyclic GMP levels during and after ischemia would collectively have an inhibitory influence on cortical excitability.

Repetitive ischemic insults are characteristic of certain pathological conditions affecting the brain circulation. The study on repeated ischemia suggests that the brain responds differently to a secondary insult and that the response is dependent on the period of recirculation. This finding may have relevance to the treatment of stroke.

The elevation of cerebellar cyclic GMP in the 20 minute ischemic group is associated with seizures in these animals. The presence of seizures may aggravate the ischemic condition and treatment with anticonvulsants which depress cerebellar cyclic GMP may be of some benefit.

Proposed Course of Project: The major emphasis is presently on the biochemical characteristics of recovery. In addition, the molecular events involved in the enormous increase in cyclic AMP in the post-ischemic period will be investigated. Similar studies on hypoxia of various degrees are planned.

Publications: Mrsulja, B. B., Lust, W. D., Mrsulja, B. J., Passonneau, J. V., and Klatzo, I.: Brain glycogen following experimental cerebral ischemia in gerbils. Experientia. 32: 732-733, 1976.

Mrsulja, B. B., Lust, W. D., Mrsulja, B. J., Passonneau, J. V., and Klatzo, I.: Post-ischemic changes in certain metabolites following prolonged ischemia in the gerbil cerebral cortex. J. Neurochem. 26: 1099-1103, 1976.

Schwartz, J. P., Mrsulja, B. B., Mrsulja, B. J., Passonneau, J. V. and Klatzo, I.: Alterations of cyclic nucleotides-related enzymes and ATPase during unilateral ischemia and recirculation in gerbil cerebral cortex. J. Neurochem. 27: 101-107, 1976.

Lust, W. D., Kobayashi, M., Mrsulja, B. B.; Wheaton, A. and Passonneau, J. V.: Cyclic nucleotide levels in the gerbil cerebral cortex, cerebellum and spinal cord following bilateral ischemia. in Tissue Hypoxia and Ischemia (M. Reivich, R. Coburn, S. Lahiri and B. Chance, eds.), Plenum Publishing Corporation, New York, 1977, pp 287-298.

Kobayashi, M., Lust, W. D. and Passonneau, J. V.: Concentrations of energy metabolites and cyclic nucleotides during and after bilateral ischemia in the gerbil cerebral cortex. J. Neurochem. 1977 (in press).

Passonneau, J. V., Kobayashi, M. and Lust, W. D.: The effect of bilateral ischemia and recirculation on energy reserves and cyclic nucleotides in the cerebral cortex of gerbils. 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02191-02 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
GABA Shunt Metabolites and Enzymes in Mouse Brain and Cells in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. V. Passonneau	Head, Sect. on Cell. Neurochem.	LNC	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

GABA shunt metabolites and enzymes have been measured in mouse brain and glial and neuronal cells in culture. GABA concentrations in brain and cultured glioma cells are comparable, whereas the neuroblastoma cells contain one-tenth as much. In general, the enzyme activities measured, GABA transaminase, succinic semialdehyde dehydrogenase and glutamate decarboxylase are much lower in the cultured cells, and GABA transaminase is absent from the glial cells. The anticonvulsant drugs, aminooxyacetate (AOA) and dipropylacetate (DPA), elevated GABA concentrations in mouse brain and neuroblastoma cells. Only DPA affected the concentrations of GABA in glioma cells. The convulsant drug, isoniazid, depresses GABA levels in brain, had no effect in glioma cells and increased GABA in the neuroblastoma cells. When pyridoxal, (a coenzyme of glutamate decarboxylase and GABA transaminase) was omitted from the medium, the drug effects were altered.

This project has been completed.

Project Description:

Objectives: To investigate the α-aminobutyric acid (GABA) shunt metabolites and enzymes in glioma and neuroblastoma cells in culture, and to study the effects of convulsant and anticonvulsant drugs.

Methods Employed: Cells are grown in a CO_2 incubator. Enzyme activities related to the GABA pathway are measured in homogenates of cells. Metabolites of the GABA shunt are measured in perchloric acid extracts of the cells. Both measurements employ sensitive enzymatic and/or radioisotope techniques.

Major Findings: The concentration of GABA in the neuroblastoma cell line is 10-fold greater than in astrocytoma cells. The respective concentrations are 6.0 and 0.55 nmoles/mg protein, as compared with 11 nmoles/mg protein in mouse brain. Glutamate concentration in both cell lines was 90 nmoles/mg protein compared with 118 nmoles/mg protein in mouse brain. The concentrations of α-ketoglutarate were 0.81, 2.29, 0.97 nmoles/mg protein in mouse brain, astrocytoma cells, and neuroblastoma cells, respectively. Glutamate decarboxylase activity in both cell lines was 2% of that in mouse brain. GABA transaminase activity was at least 10-fold greater in neuroblastoma cells than in the astrocytoma cells, and there is some question that the enzyme may be lacking entirely in the glioma cells. Such a finding would explain the higher GABA concentrations in the astrocytoma cells, since further metabolism of GABA would not be possible. Glutamate dehydrogenase activity in the astrocytoma cells was half that in mouse brain, which is 1200 nmoles/mg protein/hr. The activity in the neuroblastoma cells was only a tenth of that in brain. The activity of succinic semialdehyde dehydrogenase was 145, 15, and 30 nmoles/mg protein/ hr. in mouse brain, C-6 astrocytoma cells, and neuroblastoma cells, respectively. The effects of anticonvulsant and convulsant drugs were investigated. Aminooxyacetate, an anticonvulsant, elevated GABA concentrations in mouse brain and neuroblastoma cells, but not astrocytoma cells. Another anticonvulsant, dipropylacetate, increased GABA concentrations in mouse brain and in both cell lines. A convulsant drug isoniazid, which depresses GABA levels in mouse brain, had no effect in the astrocytoma cells, and elevated GABA in the neuroblastoma cells. The relationship of the drug effects to the enzyme activities is under investigation.

The drug effects were also assessed in the absence of pyridoxal, a coenzyme of glutamate decarboxylase and GABA transaminase. Dipropyl acetate increased GABA in the astrocytoma cells under these conditions. In the presence of isoniazid, glutamate and α-ketoglutarate were decreased and cyclic GMP increased. In the neuroblastoma cells, glutamate and α-ketoglutarate were similary decreased with no change in cyclic GMP.

Significance to Biomedical Research and the Program of the Institute: The mechanism of action of anticonvulsant and convulsant drugs has been the subject of extensive investigations. GABA, an inhibitory neurotransmitter, has been implicated as one possibility of drug action. When anticonvulsant drugs are administered to mice, the cerebellar levels of GABA increase, cyclic GMP decreases, and the threshold for seizures (electroconvulsive shock) is elevated. It is possible by using cells in culture to determine whether glioma and neuroblastoma cells are affected differently, and to isolate the site of action of the drugs.

Proposed Course of the Project:
This project has been completed.

Publications: Passonneau, J. V., Lust, W. D., and Crites, S. K.: Studies on the GABAergic system in astrocytoma and neuroblastoma cells in culture. Neurochem. Res. 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02215-02 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Localization of $(Na^+ + K^+)$-ATPase in the Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. H. Jean, Ph.D.	Visiting Associate	LNC	NINCDS
Other:	R. W. Albers, Ph.D.	Chief, Section Enzymes	LNC	NINCDS

COOPERATING UNITS (if any)

J. G. Wood, D. Anat., U. Tenn., Memphis
D. K. Morest, D. Anat., Harvard Univ. Med. School

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Enzyme Chemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	2.0	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electron microscopic localization of the $(Na^+ + K^+)$-ATPase in brain of the knive-fish has continued in collaboration with Dr. Wood More recent studies have centered upon the localization in certain astrocytic processes and in sub-lemmal cisternae of axons. In collaboration with Dr. Morest, we are exploring the use of E.M. techniques to establish the "sidedness" of the localization of ATPase antigens in electroplax membranes.

Project Description:

Objectives: To determine the location of $(Na^+ + K^+)$-ATPase within cells of the central nervous system. In particular, (1) to ascertain the relationship between the Na^+ transport system and various specialized structures of neurons; nodes of Ranvier, synapses, and glial processes; (2) to study the possible relationship of sub-lemmal cisternae to the biosynthesis of membrane structures.

Methods: Perfused brains are fixed; frozen sections are cut incubated with ATPase antisera; the ATPase antibody is stained via the indirect peroxidase method; after embedding in plastic, thin sections are prepared for examination by electron microscopy.

Major Findings: Our initial findings were described in the previous report. Our recent studies have shown the presence of $(Na^+ + K^+)$-ATPase in sub-lemmal cisternae, frequently in proximity to nodes of Ranvier. We have also observed a variable staining of presynaptic endings and of astrocytic processes surrounding synapses.

Significance: Knowledge of the localization of the active transport system for Na^+ can contribute to solving several important questions: (1) the relative extent to which astrocytes may participate in the process of controlling extracellular K^+ in the neighborhood of synapses in an important aspect in relation to the pathogenesis of seizure activity in brain; (2) the localization of a specific membrane protein at the nodes of Ranvier raises the question of how such proteins are transported and incorporated into the axolemma; (3) more detailed studies of the localization of active transport in presynaptic nerve processes could enlarge our understanding of the regulation of neurotransmitter release.

Proposed Course: Because of the departure of Dr. Jean, who has provided the antisera, the future of this project is uncertain. Dr. Wood has expressed an interest in extending this work to achieve a more definitive picture of the relation of sub-lemmal cisternae to plasma membrane biosynthesis.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02254-01 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Use of Neurological Grafts to Repair the Injured Peripheral or Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. A. Zalewski Act. Sect. Head, Neural Development and Regeneration LNC NINCDS

COOPERATING UNITS (if any)

W. K. Silvers, Department of Human Genetics, University of Pennsylvania
H. G. Goshgarian, Guest Worker, Post-Doctoral from Paralyzed Veterans of Ameri

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Neural Development and Regeneration

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the use of a peripheral nerve allograft to provide a neurilemmal system (i.e., Schwann cells and their basement membranes) which might promote the regeneration of host nerve fibers at sites of injury to the peripheral or central nervous system. Initial studies are being performed to determine whether an immune response occurs to a nerve allograft and what influence tissue histocompatibility has on allograft survival. The results in rats show that a nerve allograft is antigenic and that grafts differing with respect to major histocompatibility antigens are rejected whereas grafts with minor antigens survive at the five weeks period the grafts were studied. Other results demonstrated that allografts (ganglia were used) with major or only minor histocompatibility antigens are rejected in the spinal cord. This demonstrates that immune mechanisms are operative in the spinal cord and that the central nervous system is not a privileged transplantation site.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: The purpose of this project is to determine the possible use of a peripheral nerve allograft to provide a neurilemmal system (i.e., Schwann cells and their basement membranes) which might promote the regeneration of host nerve fibers at sites of injury to the peripheral or central nervous system. There is a need to explore the use of an allograft (i.e., a graft from a genetically different animal of the same species) because an autograft (i.e., a graft taken from one site and used in another site of the same animal) of sufficient size, quantity, or anatomical structure (i.e., the arrangement of nerve fascicles within a nerve) may not be available and because removal of an autograft in itself causes some neurological deficit. The idea to use a nerve allograft is not new, but results obtained using them are confusing or conflicting (Bucko and Steinmuller, Plast. Recon. Surg., 1974, 53: 326-331). There seems to have been little regard given to the influence of histocompatibility in selecting donor allografts and this omission could be significant. We know from our studies with allografts of ganglia that graft histocompatibility is important because ganglia allografts were rapidly rejected (i.e., neurons and Schwann cells) when donors and recipients differed in major histocompatibility antigens whereas they survived (at least for the duration the experiment was performed) when only minor histoincompatibilities prevailed (Zalewski, Exp. Neurol., 1971, 33: 576-583). It should be pointed out that the type of major antigens present on an allograft can be detected this way. Moreover, minor antigens may not express themselves similarly in all grafts as evidenced by the rapid rejection of skin but the indefinite survival of kidney when these organs are transplanted across minor histocompatibility barriers (Sakai, Transplantation, 1969, 8: 882-889). It would be desirable therefore to reexamine the use of peripheral nerve allografts by employing animals of known major and minor histocompatibilities. Furthermore, nerve allografts should be examined in immunologically tolerant recipients (i.e., animals made unresponsive to allograft antigens because suppressor lymphocytes or blocking antibodies were induced in them by their neonatal exposure to donor allograft antigens). Tolerant animals should accept nerve allografts which is the definitive way to demonstrate that loss of a nerve allograft in a non-tolerant animal, should it occur, is due to an immunological rather than non-immunological (i.e., vascular) cause. The importance of studying the immune reaction to nerve allografts may also have implications with regard to repair of the injured spinal cord. Kao reported that nerve autografts into the transected spinal cord of dogs helped restore the dogs' walking ability (Kao, Exp. Neurol., 1976, 53: 815-829). More recently this investigator provided histological evidence of regenerated nerve fibers in the graft which could explain his prior success of cord repair in dogs (Kao, Exp. Neurol., 1977, 54: 591-615). Of course the autografts used caused other defects in the dog which is why an allograft should be tested. Accordingly, it will be necessary to determine if an immune reaction occurs to a neurological allograft in the spinal cord, what influence tissue histocompatibility has on an allograft, and whether tolerance can be induced to a spinal cord allograft.

Methods Employed: Nerve allografts will be performed using isogenic strains of Lewis (LE), Fischer (FR) and Brown Norway (BN) rats. LE nerve grafts will be tested in normal BN rats and in BN rats made immunologically tolerant to LE histocompatibility antigens. LE nerve grafts will also be examined after transplantation to normal FR or LE-tolerant FR rats. The success of nerve allografts in the peripheral nervous system will be revealed by determining whether host motor and sensory nerve fibers can regenerate through the nerve allograft and reinnervate muscle and taste buds respectively. Specifically, a common peroneal nerve allograft will be used to reinnervate the tibialis anterior and extensor digitorum muscles of the leg while a glossopharyngeal nerve allograft will be used to reinnervate and induce taste buds in the vallate papilla of the tongue. A common peroneal nerve allograft will also be used in the injured cervical spinal cord to try to encourage respiratory fibers to connect with phrenic motor neurons and restore hemidiaphragmatic movement. Neurohistologic, histochemical, and electromyographic techniques will be used to correlate anatomical regeneration with functional recovery.

Major Findings: The animals to be used for the nerve allograft study are currently undergoing the appropriate surgery. However, the results of two other preliminary allograft experiments are pertinent. Nerve allografts have been transplanted to the anterior chamber of the eyes of rats to determine if nerve allografts are antigenic. The data demonstrate that 1 cm long tibial nerve grafts from LE rats in BN eyes incite an intense immune response and that neurilemmal (Schwann) cells are rejected by 35 days. On the other hand, an LE nerve graft to an FR eye causes a mild response at this time and neurilemmal cells survive. Autografts of nerve did not evoke any immune reaction and neurilemmal cells were present. Another study was done in which an allograft of ganglion (sensory vagal nodose) was implanted into the spinal cord. This was done to determine if the spinal cord is competent to develop an immune response. The results demonstrated that LE ganglia were rejected (i.e., no neurons or neurilemmal cells were present) at 60 days in the BN spinal cord. Autografts of ganglia survived in the cord and neurons and neurilemmal cells were present. We are awaiting the results of the fate of LE ganglia implanted into the cord of LE-tolerant BN rats and LE ganglia into normal FR or LE-tolerant FR rats.

Significance: The results with nerve allografts in the eye indicate that the cells in it are antigenic and evoke an immune response. Histocompatibility seems to influence graft survival as the graft in the more compatible LE to FR rats survived whereas the more incompatible LE to BN graft was rejected. This result may mean that host nerve fibers may be able to regenerate through a more histocompatible nerve allograft when nerve repair is performed on the common peroneal nerve in situ. It will be interesting, however, to see what happens to the function of host nerve fibers that have regenerated through a nerve allograft if the allograft segment in LE to FR rats is rejected at a much later time. The result demonstrating that allografts of LE ganglia are rejected in the spinal cord of BN rats indicates that immune mechanisms are operative in the spinal cord. It will now be important to see if tolerance induction in BN rats permanently prevents the rejection of LE grafts in the spinal cord as it did for LE ganglia in the eyes or muscles of LE-tolerant BN rats (Zalewski and Silvers, 1977, manuscript in preparation).

Proposed Course of Project:

1) Determine the fate and function of nerve allografts in normal and tolerant animals.

2) Determine if the injured spinal cord in the immunologically deficient nude mouse regenerates without the aid of a graft. This will test the hypothesis that an autoimmune disease to the central nervous system develops after injury and prevents regeneration (Berry and Riches, Brit. Med. J.), 1974, 30: 135-141).

3) Determine if a xenograft (i.e., graft from another species of spinal cord promotes central regeneration in the nude mouse when the xenograft is taken from those species of amphibians which readily reconstitute their injured cord. Such xenograft studies are possible in the nude mouse because this animal is congenitally athymic and lacks mature T-lymphocytes which are necessary for graft rejection or the development of autoimmune disease (see 2 above).

Publications:

1. Zalewski, A. A. and Silvers, W. K.: The fate and functional capacity of neonatal neurons in allografts of ganglia in normal and immunologically tolerant rats. Exp. Neurol. 52: 507-514, 1976.

2. Zalewski, A. A. and Silvers, W. K.: A differential survival of ganglia and skin allografts in adult rats neonatally treated with bone marrow cells. Transplantation 23: 106-109, 1977.

3. Zalewski, A. A. and Silvers, W. K.: The long-term fate of neurons in allografts of ganglia in Ag-B-compatible normal and immunologically tolerant rats. J. Neurobiol. 8: 207-215, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02255-01 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
GABA and Nucleotide Metabolism in Brain Mitochondria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Rodichok, M.D., Research Associate	LNC	NINCDS
	R. W. Albers, Ph.D., Head, Section Enzymes	LNC	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Enzyme Chemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	2.0	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of these studies is to determine the inter-relationships between substrates for brain mitochondria and the turn-over of mitochondrial nucleotides. In particular, we are exploring a possible regulatory role of γ-aminobutyrate in mitochondrial energy metabolism. We have established that substrate level phosphorylation has a special role rephosphorylating adenylic acid in brain as well as liver. Methodology and baseline values for the levels and turnover rates of brain mitochondrial nucleotide have been determined.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine the kinetic pattern of phosphorylation of nucleotides in brain mitochondria as influenced by different energy substrates. In particular, to assess the hypothesis that the so-called "GABA shunt" pathway in brain may serve to regulate specific phosphorylation pathways dependent upon guanine nucleotides.

Methods: Brain mitochondria are prepared by differential centrifugation. Substrate oxidation is measured polarigraphically. Nucleotides are separated by high pressure liquid chromatography. When appropriate, a rapid mixing device is employed to obtain reaction times as short as 80 msec. Phosphorylation of nucleotides is assessed by measuring the rate of incorporation of radioactive ortho-phosphate into the individual nucleotides after HPLC separation. The nucleotide pattern is evaluated by parallel measurements of optical observance, the distribution of ^{32}P and the distribution of ^{3}H in the separated nucleotides. Tritiated nucleotides are used as internal standards to quantitate the recovery of each component. A computer program is used to integrate the area of the chromatographic curves and to resolve overlapping components.

Major Findings: Baseline levels for nucleotides in brain and liver mitochondria have been established. We have confirmed a previously published report that GTP generated by substrate-level phosphorylation in liver mitochondria is involved in the rephosphorylation of adenylic acid. We have tentatively demonstrated a similar pathway in brain.

Proposed Course: We are investigating the initial kinetics of nucleotide phosphorylation to more firmly establish the product-precursor relationship between GTP and ADP of brain mitochondria. In addition the efficacy of various TCA cycle intermediates in supporting this process will be evaluated. In particular, we shall examine the possible regulatory role of γ-aminobutyrate and succinic semialdehyde on the kinetics of nucleotide phosphorylation. Exploration of possible pathways for the generation of adenylic acid by brain mitochondria is also proposed.

Significance: The "GABA shunt" is a pathway unique to brain which constitutes a potential bypass of the substrate-level phosphorylation of GDP to GTP. The unique role of GTP in liver mitochondria appears to be rephosphorylation of adenylic acid. Although we have demonstrated the formation of adenylic acid within brain mitochondria, the reactions responsible for this are not as yet known. This constitutes a relatively unknown segment of brain energy metabolism that deserves further study.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 NS 02256-01 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolic Profiles in Normal and Diseased Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDS
OTHER:	W. D. Lust	Research Pharmacologist	LNC	NINCDS
	F. de Azeredo	Visiting Fellow	LNC	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are in progress on retinal metabolism in whole retinas and frozen-dried sections of retinal layers. The effects of "spreading depression" on high-energy phosphate compounds and cyclic nucleotides is being investigated; ATP, P-creatine, cyclic GMP, and cyclic AMP have been measured in the whole chick retina after stimulation. Preliminary studies on frog retina of tricarboxylic cycle intermediates, glycolytic intermediates, cyclic nucleotides and high-energy phosphate compounds have been made.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine the alterations in metabolism of the retina during "spreading depression". To determine the distribution of metabolites, putative transmitters, cyclic nucleotides in the layers of frozen-dried frog retina in normal animals and animals treated with drugs to destroy the rod outer segments.

Methods Employed: In the case of the whole retina, the chick is decapitated, the eyes are removed and cut in half. After incubation at 35-37°, the retina can be removed intact with a pair of forceps. The retinas are transferred to teflon racks in which depressions are drilled for experimental chambers. The retina is stimulated by touching with a fine glass filament to cause "spreading depression". At appropriate intervals the retinas are frozen in liquid nitrogen, extracted with perchloric acid, and the extracts stored until analyzed.

For the studies on retinal layers, frogs are decapitated into liquid nitrogen, the heads frozen and stored at -70° until dissection. The eyes are dissected at -20° and the whole eyeball removed. The eyeball is mounted on a specially designed holder, and sectioned at 10 μm at -25° in a cryostat. The sections are vacuum-dried at -40°, after which they can be stored at -20° under vacuum. For microdissection, the samples are brought to room temperature under vacuum, removed, and the retinal layers (approx 0.1 μg) dissected with micro instruments using a low-power dissecting scope. The microchemical analyses are performed in oil wells using volumes of 0.05 to 0.5 μl.

Major Findings: In the studies of spreading depression, ATP and P-creatine have been found to decrease in tandem, falling to 66% of control levels 30 sec after stimulation, and reaching 50% at 3 min.

Both cyclic AMP and cyclic GMP are depressed after stimulation, the first decrease seen at 2 min after stimulation (to 66% of cAMP and 50% of cyclic GMP control values). At the latest times studied (3 min); the cyclic nucleotides are still decreasing, although slowly.

In the studies of the layers of frog retina, no major findings can be reported as the work is still preliminary in nature.

Significance to Biomedical Research and the Program of the Institute: Because of the layered structure of the retina, and the concentration of mitochondria in the inner segment (layer 2b) the question of sequestration of certain metabolites of the citric acid cycle in these organelles may be approached. It is an ideal place to study whether ADP, for example is limited exclusively to the mitochondria.

The location of key metabolites such as ATP, ADP, 5'AMP, and citrate will help elucidate metabolic controls.

The function of cyclic GMP in the retina is not yet understood. It is as high as 600 pmoles/mg protein in the rod outer segments (100 x greater than the tissue with the next highest concentration, the cerebellum). The amount of cyclic GMP is increased in dark-adapted retinas as much as three-fold. Most of this work has been done in isolated rod outer segments. Our techniques provide a unique possibility to study the *in vivo* effects of light, to compare normal retinas and those in which the rods have been destroyed by drugs and/or congenital retinopathies. Such investigations may help elucidate the mechanism of photoactivation of the retina, and the biochemical pathology of retinopathies.

Proposed Course of Project: The preliminary work in retinal layers will be developed further. Both normal and diseased retinas will be studied in an attempt to learn the biochemical nature of retinopathies.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02257-01 LNC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemistry of Experimental Seizures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. D. Lust	Research Pharmacologist	LNC	NINCDS
OTHER:	J. V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDS
	D. W. McCandless	Staff Fellow	LNC	NINCDS
	Gretchen Feussner	Chemist	LNC	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Section of Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study is being made of chemically- or electrically-induced seizure activity in mouse brain. Work to date has indicated that elevated cyclic GMP concentrations in the cerebellum are associated with many types of seizures, and that when convulsions are suppressed by drugs, cyclic GMP is decreased. In addition, increased GABA concentrations may be involved in anticonvulsant action. Studies on whole mouse brain indicate that a major locus of action of phenytoin is in the cerebellum. Microdissection and microanalytical procedures are being used to analyze cerebellar layers after electroshock and in the presence of phenytoin.

PHS-6040
(Rev. 10-76)

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